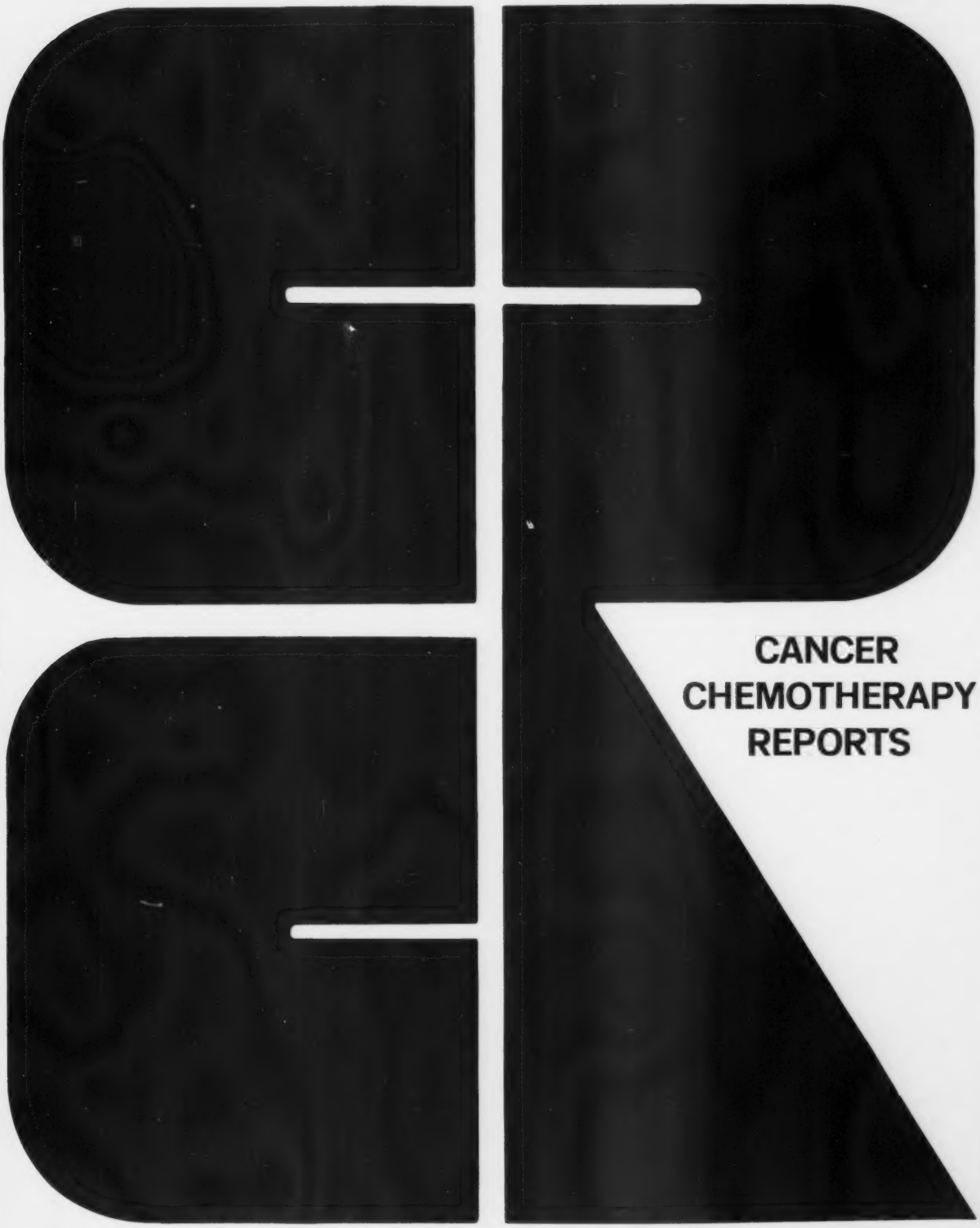

CANCER CHEMOTHERAPY REPORTS

Part 1

Mar/Apr 1975
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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

LETTERS

Hyperthermia as an Adjuvant in the Treatment of Osteogenic Sarcoma

A study of literature reviews on the use of hyperthermia in the treatment of malignancy (1,2)[1] leads us to conclude that hyperthermia could be a valuable adjuvant to chemotherapy in the treatment of osteogenic sarcoma.

In their experience with osteogenic sarcoma, Jaffe et al[2] observed indications that in the majority of patients, hematogenous pulmonary micrometastases are present at the time of diagnosis. Furthermore, in those cases where local control of the disease was gained by amputation of the extremity, pulmonary metastases and death still resulted in over 80% of the patients within 12–18 months. They further report that in those cases where local control was achievable, adjuvant chemotherapy reduced the occurrence of pulmonary metastases significantly, and that this systemic approach "delays relapse and may provide definitive treatment in some patients with osteogenic sarcoma."

We are especially interested in those patients, most of whom are young, who at diagnosis already have lesions beyond the extremities and, therefore, are not subject to local control of the disease by amputation. We believe these patients may benefit from a regimen of thermotherapy/chemotherapy.

Clinical trials could be designed for whole-body hyperthermia in a manner similar to the techniques of either Henderson and Pettigrew (3), who used warmed anesthetic gases for a patient encased in wax, or von Ardenne (4), who immersed the patient in a temperature-controlled water bath. This thermotherapy would be followed by a regimen of chemotherapy similar to that reported by Jaffe et al.[2] The clinical technique of Henderson and Pettigrew is at present being used and refined by Dickson (5) and probably others.

We suggest that a preferred clinical technique for those patients where the primary lesion is in a limb would be a procedure similar to the technique used by Stehlin (6).[3] This technique appears very successful for the treatment of melanomas with primary lesions in the extremities, and involves perfusion of the extremity which has or had the primary lesion with a heated blood-chemical solution. In those cases where regression was obtained in the extremity, regression of the metastases in the trunk also occurred. Dickson and Murkle (7) observed the same phenomenon of metastases regression when established VX-2 carcinomas in rabbits were treated by immersing the tumor-bearing limb in warmed water. Cavaliere et al (8) observed a reduction in the number of deaths from metastases in patients with advanced melanomas and sarcomas who were treated by regional hyperthermic perfusion.

The regression of the metastases beyond the hyperthermically treated region is of special significance and may well be relatable to the metastases resulting from osteogenic sarcoma (9–12). Dickson and Murkle logically examined this metastatic regression and hypothesized that "stimulation of the immune system by tumor breakdown products following local heating would be consistent with clinical observations." They further examined the possibilities that the time/temperature combinations were too small and/or that a depression of the immune system accounted for the observed lessened response to whole-body thermotherapy.

Without doubt, improvements in the proposed regimens of thermotherapy/chemotherapy can be defined by others and by clinical trial. The

[1] Allan BD, and Norman RL. Water structural change: a key to the understanding of malignancy. Presented at the 24th Annual American Chemical Society Southeastern Regional Meeting, Birmingham, Ala, Nov 2–4, 1972.

[2] Jaffe N, Frei E III, Traggis D, et al. Adjuvant methotrexate-citrovorum factor treatment of osteogenic sarcoma. Harvard Medical School and Harvard School of Public Health, Boston, Mass.

[3] Stehlin JS. Clinical studies of regional (limb) hyperthermia in melanoma. Presented at the National Cancer Institute, National Institutes of Health, Bethesda, Md, Apr 5, 1974.

present knowledge and state-of-the-art in chemotherapy and thermotherapy lead us to recommend clinical trials of this therapy combination in selected osteogenic sarcoma patients.

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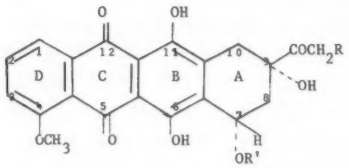
September 9, 1974

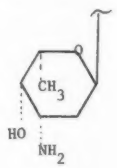
Another Hypothesis Concerning the Antitumor Activity and Cardiotoxicity of Daunorubicin (NSC-82151) and Adriamycin (NSC-123127)[1]

Adamson (1) recently suggested that the cardiotoxicity of adriamycin *(1a)* and daunorubicin *(1b)* can be attributed to the daunosamine *(2)* portion of the molecules, and that compounds in which the aglycones bear an amine or alkylamine group might show antitumor activity with no cardiotoxicity. The results of Müller et al (2) with alkylaminoanthraquinones support Adamson's ideas.



1a R=OH; R' = daunosamine (2)

1b R=H; R' = daunosamine (2)



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I should like to present another (but not conflicting) suggestion. The anticancer activity of adriamycin and daunorubicin is probably connected with their ability to form intercalated complexes with DNA (3–10). The formation of the complexes appears to involve the amino sugar group and the quinone chromophore of *1a* and *1b*. Mhatre et al (11,12) and Yesair et al (13) have provided evidence that the cardiotoxic properties associated with *1a* and *1b* are related to the formation of aglycones in the heart, and that the cleavage to the aglycones is more rapid for *1a* and *1b* than for *N*-acetyldaunomycin, which does not interact well with DNA and does not show as much anticancer activity as *1b*. I suggest the following explanation for the effect of the interaction of *1a* or *1b* with DNA on the stability of the glycoside link. The structure of *1b* determined by X-ray crystallography (14) shows that the hydroxyl group at C_6 is strongly hydrogen-bonded to the quinone carbonyl group at C_5. Since intercalation involves the quinone system, it is possible that the hydrogen bond between O_5 and O_6 is no

[1] Daunorubicin: CAS reg. No. 23541-50-6; daunomycin; rubidomycin (NSC-83142).

longer present when ***1b*** is complexed with DNA. The C_6 hydroxyl group would then be available to hydrogen-bond with the C_7 oxygen and this would catalyze the breakdown of the glycoside link. If this theory is true, removal of the C_6 hydroxyl group should give a compound which is less liable to break down to the aglycone on complexing and therefore (if Mhatre's ideas are correct) should be less damaging to the heart. I suggest that 6-deoxy ***1a*** or ***1b*** would be worth making and testing. My research group is working on this problem. In addition, amino-containing groups linked by an ester or ether to the aglycone at C_7 would be cleaved less readily than ***1a*** or ***1b*** and so would also be worth investigation.

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November 7, 1974

Further Discussion of Ineffective Single Agents in Combination Chemotherapy

According to the Bunn dictum (1), "when an agent is ineffective alone, its use in combination trials is generally unwarranted." There is at least one category of combinations potentially valuable in chemotherapy which would be eliminated by adoption of this rule. This would include the use of combinations in which one substance, nontoxic in itself, is used to minimize the degradation or metabolism of another agent which alone has shown some chemotherapeutic efficacy. If one follows the Bunn rule, then we must not add a nontoxic substance to form a combination which can minimize the extensive excretion or degradation of an effective agent. Many scientists engaged in studies on how to improve the efficacy of promising chemotherapeutic agents would be pleased if Dr. Bunn stated that he and the Division of which he is a member did not mean to preclude the use of such combinations.

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November 15, 1974

DNA Complexes of Daunorubicin (NSC-82151) and Adriamycin (NSC-123127)

Referring to the recent report by D. W. Henry (1), we wish to draw attention to the fact that the pharmacologic and therapeutic properties of the DNA complexes of daunorubicin and adriamycin critically depend on the nature of the DNA used and on the method of preparation of the complex.

We have found that NSC-169534 (adriamycin-DNA)[1] used by Henry differs significantly from the adriamycin-DNA prepared in our laboratory (2). After intravenous (iv) injection of the NSC preparation into mice, the concentration of the drug in the blood hardly rises higher than it does after injection of free adriamycin, and the concentration falls as fast as with free adriamycin. In contrast, when our DNA complex is injected the drug concentration in the blood reaches a much higher level and declines considerably more slowly.

When tests were made using DBA/2 mice inoculated iv on Day 0 with 10^4 L1210 leukemia cells and then given four consecutive iv injections of adriamycin at the dosage of 7 mg/kg/day on Days 1–4, the following results were obtained. In the controls, the median survival time was 6 days; no animals survived. In the group receiving free adriamycin, the median survival time was 16 days and two of ten animals survived more than 40 days. In the group treated with the NSC-169534 DNA complex, the median survival time was 23 days and there were four long-term survivors out of ten animals treated. In the group treated with our DNA complex, the median survival time was more than 40 days and eight of ten animals survived.

In view of these findings, it is likely that the results reported by Henry with the DNA complexes of adriamycin and daunorubicin[2] fall below what can be obtained with the best preparations of these complexes.

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December 3, 1974

[1] Adriamycin-DNA: NSC-169534; nucleic acid, deoxyribo-, sodium salt, compd. with (8*S-cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride.

[2] Daunorubicin-DNA: NSC-169533; nucleic acid, deoxyribo-, sodium salt, compd. with (8*S-cis*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)-oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride.

COMMENTARY

Antiviral Chemotherapy as an Indicated Adjunct to Cancer Chemotherapy[1,2]

Frank M. Schabel, Jr.[3,4]

ANTIVIRAL DRUGS FOR ACUTE INFECTIONS ASSOCIATED WITH CYTOTOXIC ANTICANCER CHEMOTHERAPY

Rational searches for antiviral and anticancer drugs have had a common base throughout their somewhat parallel history. Viruses are highly evolved obligate intracellular parasites, depending upon their host cells for most of the building blocks of self-replication and usually retaining to themselves only their genetic function. They are classified as DNA or RNA viruses, depending on the primary and often nearly exclusive nucleic acid content of each, and a number of infectious crystalline viral nucleoproteins have been isolated. Thus, structural analogs of natural purines and pyrimidines as inhibitors of de novo nucleic acid synthesis have been logical candidates to test for antiviral activity, and, since tumor cells must synthesize new DNA and RNA prior to cell division, most of the same analogs of nucleic acid precursors have been studied for anticancer activity. As a tribute to Mother Nature's highly ordered world, and as we might have expected, very few antiviral agents have been shown to have usable antitumor activity and very few antitumor agents have been shown to have usable antiviral activity. Despite this lack of common activity, a number of useful antiviral drugs have been discovered and an even larger number of useful anticancer drugs are currently known.

[1] Received Aug 23, 1974; accepted Sept 26, 1974.

[2] Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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Severe and often life-threatening virus infections commonly occur in cancer patients, particularly in those with malignant disease of the reticuloendothelial system (RES) with associated depression of humoral and cellular immunity (1–4), and in patients with tumors other than those of the RES who are receiving immunity-suppressing antitumor drugs and adrenal corticosteroids (1,3). Increased incidence and severity of virus infections are also seen in organ-transplant patients treated with immunosuppressive drugs (2,5). The most important and common of these virus infections in cancer patients are caused by a number of DNA viruses (herpes simplex, herpes zoster, varicella, cytomegalovirus, and vaccinia). One RNA virus infection (measles) is also a recognized clinical problem in cancer patients (1–3).

Most clinically useful anticancer agents have been examined for antiviral activity and, with the possible exception of arabinofuranosylcytosine, none have been shown to have clinically useful antiviral activity (6–9). However, several antiviral agents are known which have been clearly or provisionally demonstrated to have useful clinical activity against all of the DNA viruses of clinical importance in the immunosuppressed (tumor or transplant) patients mentioned above. Among these antiviral drugs are:

Idoxuridine: clearly active against localized and possibly active against generalized herpes simplex (8,9), generalized herpes zoster (8,9), cytomegalovirus (8), and vaccinial keratitis (8).

Arabinofuranosylcytosine: active against localized and generalized herpes simplex (8,9) and possibly active against herpes varicella

(9), although this latter activity has been questioned (10).

Arabinofuranosyladenine: active against localized and generalized herpes simplex (7,9), localized and generalized herpes varicella (7), localized vaccinia (7), and cytomegalovirus infections (7).

Methisazone: active against variola (8) and local and generalized vaccinia (8,9).

No clinically useful drug against measles in man has been described, although Bauer has suggested that 6-azauridine, currently under study, may be promising against measles in man (8). It is of interest to note that 6-azauridine has also been reported to be active against variola, herpes simplex, and herpes varicella in man (6).

Thus, while we currently have available antiviral drugs with clinical usefulness against the major virus diseases afflicting tumor patients, none are ideal (ie, have a broad antiviral spectrum, are nontoxic to normal cells and tissues, are easy to administer, etc). As aggressive and effective drug treatment of cancer continues to improve in therapeutic utility through the use of more active drugs and combinations of drugs for more extended treatment periods, we may expect the serious side effects, including increased incidence of virus infections, to increase. These possibilities (probabilities) are a clear indication for renewed and increased pragmatically oriented searches for more and better antiviral drugs, particularly in view of the practical success in antiviral chemotherapy cited above.

ANTIVIRAL DRUGS FOR USE AGAINST ONCOGENIC VIRUSES

If we (*a*) assume viral etiology of human tumors (a persuasive concept when considered in the light of numerous examples of viral oncogenesis in lower animals) (11,12), (*b*) disregard the "oncogene" theory (13), and (*c*) assume viral oncogenesis to occur after horizontal transmission of a specific intact and independently functional virus to one or more intact normal cells,[5] then in vivo inhibitors of virus growth may well be required to control reinduction of virus-induced tumors once the primary clinical episode has been "cured" by surgery, radiation, drugs, or multimodality treatment.[6] Data collected during and after effective chemotherapy of spontaneous leukemia-lymphoma in AKR mice have been interpreted as indicating a very high probability of having achieved drug cure of the cellular phase of the disease, only to have all animals ultimately die of the pathologically classical disease (presumably virus reinduced), often as much as several months after apparent drug cure and well beyond the anticipated maximum interval to death from one viable tumor cell surviving drug treatment. Additionally, we have been unable to detect by sensitive bioassay any viable tumor cells in a significant number of animals at the end of drug treatment (14), indicating likely "cell cure."

Broad-spectrum antiviral agents, active against both RNA and DNA viruses, including known and likely oncogenic viruses, are well known. Nearly 10 years ago, I cited the reported activity of both cycloheximide and noformicin against both RNA and DNA viruses (15), and Bauer, Apostolov, and Selway reported the activity of methisazone against a number of RNA and DNA viruses, including adenoviruses (16,17), reported earlier by Trentin, Yabe, and Taylor to be oncogenic in hamsters (18). Ribavirin has been reported to have antiviral activity against a wide variety of DNA and RNA viruses, including a number of known and suspected oncogenic viruses (19).

A number of agents with widely varying chemical structures have been reported to be active in vitro against an impressive list of

[5] This phenomenon is well established and widely recognized, both in naturally occurring infections with oncogenic viruses in domestic animals and with laboratory transmission of oncogenic viruses in experimental animals (11,12).

[6] I have regarded long-term prophylactic treatment of normal individuals (no clinical history of cancer) with antiviral drugs to be a practical impossibility, since to be effective it would have to be given to everyone throughout life to protect a population only 25% of which is at risk. Such a prophylactic drug would have to be effective against all oncogenic viruses and completely nontoxic at effective dose levels.

TABLE 1.—Antiviral activity of selected compounds against murine oncogenic viruses in cell culture

Compound	Virus	Reference No.
Purine and pyrimidine nucleoside analogs		
3-Deazauridine	Gross MLV*	20
Arabinofuranosyladenine	Gross MLV	21
	Rauscher MLV	†
Ribavirin	Gross MLV	†
	Rauscher MLV	‡
	Moloney MSV§	‡
Polynucleotides		
Polyadenylic acid (poly A)	Gross MLV	†
	Moloney MLV	22, 23
Poly(2'-0-methyladenylic acid)	Moloney MLV	24
	Moloney MSV	24
Antibiotics		
Rifamycin SV	Gross MLV	25
	Rauscher MLV	26
	Moloney MSV	26, 27
Dimethylbenzyl-rifampicin	Gross MLV	25
	Rauscher MLV	26
	Moloney MLV	28
	Moloney MSV	27, 29

*MLV = murine leukemia virus.
†Shannon WM. Personal communication.
‡Chirigos MA. Personal communication.
§MSV = murine sarcoma virus.

murine oncogenic viruses, including Gross leukemia virus. Selected examples are listed in table 1. Among these, arabinofuranosyladenine is of great interest since it is active against herpes simplex virus, and a growing body of evidence deeply implicates herpes simplex (especially type II) in tumor induction in man (30).

Arabinofuranosyladenine is active in vitro against RNA oncogenic viruses (table 1) and has recently been reported active against Shope fibroma virus (a DNA virus) in vivo (31).

Marked selective inhibition of viral DNA synthesis over host cell DNA synthesis occurs in herpes simplex virus-infected human (KB) cells in culture treated with arabinofuranosyladenine (32). DNA synthesis in HEp-2 cells in culture that are infected with herpes simplex virus and treated with arabinofuranosyladenine is significantly inhibited, while no inhibition of DNA synthesis has been seen in similarly treated cells that are not infected with herpes simplex virus (33). This selective intracellular inhibition of viral DNA synthesis occurs at identical drug concentrations, since viral and cellular DNA syntheses are taking place in the same intracellular milieu (32). This is true biochemical selectivity, clearly free of the reservations of "devil's advocates" who often ascribe reported biochemical selectivity to differences in membrane transport, catabolic enzyme activity, etc.

EPILOG

What does all this have to do with our current problems? In my judgment, the promise of developing improved antiviral drugs for the treatment of acute viral infections commonly seen in immunosuppressed cancer patients is very real. Additionally, the scientific data attesting to the vulnerability of both RNA and DNA oncogenic viruses, in particular those

which are horizontally transmitted and may reinduce neoplasia after cellular cure of clinical disease by surgery, radiation, and/or drugs, to broad-spectrum antiviral agents indicate to me that we may anticipate the control of viral reinduction of tumors in patients (if such should be shown to occur) who have been cured of the cellular form of their disease. The tools for such studies are at hand, and although they are perhaps still crude, they can be improved. Candidate antiviral agents are currently available, and better ones will surely be found if we aggressively seek them. What we need are energetic and imaginative investigators (and we have many) who are supported and encouraged to apply and expand existing knowledge to these very important problems in cancer and virus chemotherapy.

LIST OF COMPOUNDS

Arabinofuranosyladenine: NSC-404241; CAS reg. No. 5536-17-4; adenine, 9-β-D-arabinofuranosyl-; ara-A

Arabinofuranosylcytosine: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C; cytosine arabinoside

6-Azauridine: NSC-32074; *as*-triazine-3,5(2*H*,4*H*)-dione, 2-β-D-ribofuranosyl-

Cycloheximide: NSC-185; CAS reg. No. 7006-21-5; glutarimide, 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-; actidione

3-Deazauridine: NSC-126849; CAS reg. No. 23205-42-7; 2,4(1*H*,3*H*)-pyridinedione, 1-β-D-ribofuranosyl-

Dimethylbenzyl-rifampicin: NSC-143414; CAS reg. No. 13292-47-2; 2,7-(epoxypentadedeca[1,11,13]trienimino)naphtho[2,1-*b*]-furan-1,11(2*H*)-dione, 8-[*N*-(4-benzyl-2,6-dimethyl-1-piperazinyl)formimidoyl]-5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-, 21-acetate, *cis*-

Idoxuridine: NSC-39661; CAS reg. No. 54-42-2; uridine, 2'-deoxy-5-iodo-

Methisazone: NSC-69811; CAS reg. No. 1910-68-5; indo-2,3-dione, 1-methyl-, 3-(thiosemicarbazone); Marboran

Noformicin: NSC-72942; 2-pyrrolidinecarboxamide, *N*-(2-amidinoethyl)-5-imino-, sulfate, hydrate (4:4:3), DL-

Ribavirin: NSC-163039; CAS reg. No. 36791-04-5; 1*H*-1,2,4-triazole-3-carboxamide, 1-β-D-ribofuranosyl-; Virazole

Rifamycin SV: NSC-133100; CAS reg. No. 6998-60-3; 2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-*b*]furan-1,11(2*H*)-dione, 5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-, 21-acetate

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Estimation of Tumor Cell Kill From Gompertz Growth Curves [1,2,3,4]

Harris H. Lloyd [5,6,7]

SUMMARY

The Gompertz function has been found to mathematically describe several types of biologic growth, including the growth of transplantable animal tumors. If it is assumed that treatment (chemotherapy, surgery, x-irradiation) of tumors causes cell death in a relatively short interval and that the surviving cells begin to repopulate immediately, producing tumor growth according to the Gompertz function before treatment, then the displacement in time between the two growth curves may be quantitatively related to tumor cell kill. This theory is developed and compared to bioassay values. It is also shown that tumor cell kill may be determined graphically without the necessity of computer-generated estimates of the Gompertz parameters.

[Cancer Chemother Rep 59:267–277, 1975]

Benjamin Gompertz (1) first expressed his "law of human mortality" in 1825. Since its presentation, the Gompertz function has not only been used to describe mortality data, but also biologic growth. In particular, Gompertz functions have been found for the growth of the human fetus (2,3), animals (4–8), man (9), individual organs (10,11), transplantable animal neoplasms (12–15), and also for animal embryonic growth (3). The mathematical properties of the Gompertz function have been described in detail (16–18). Recently it has been shown, within the bounds of certain assumptions regarding biologic growth, that the Gompertz function is the only function consistent with the allometric law (18).

It has been recognized (19) that when solid animal neoplasms are treated by chemotherapy the extent of tumor mass regression is a poor indicator of actual decrease of tumor cell viability. In fact, the decrease in viability, as determined by bioassay, may be a factor of several decades greater than the regression of tumor size. It was suggested that a better estimate of tumor cell viability, and therefore the effectiveness of chemotherapy, could be obtained by extrapolating the recurring growth curve back to the time of treatment (19). However, this technique requires sufficient growth data on the recurring tumor to make the extrapolation reliable. It has also been recognized (20) that the "time to recurrence" after treatment must be

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[4] All drugs used in this study were supplied by Drug Research and Development, DCT, NCI.

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[6] The author gratefully acknowledges the cooperation of Mrs. Linda Simpson-Herren, who provided the cell-cycle data, and Dr. Daniel P. Griswold, who provided the bioassay data.

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related to the fraction of cells that survive therapy. The fewer the number of viable cells remaining after therapy, the longer the duration required for the tumor to return to the size at treatment.

This report presents a method of using Gompertz growth curves to estimate the extent of tumor cell kill following therapy. The theory is applicable to single-dose chemotherapy, x-irradiation, and surgery, but may be extended to include fractionated x-irradiation or multiple-drug and multiple-dose chemotherapy. The Gompertz function will be described in sufficient detail to make the theoretical presentation meaningful. Two procedures for computing cell kill will be described. One requires a computerized determination of the Gompertz parameters for a particular set of tumor growth data; the other is graphical and requires only an estimate of the asymptotic size of the untreated tumor. Cell kill determined from Gompertz growth curves is then compared to cell kill determined by bioassay for several animal neoplasms. Finally, a brief error analysis is presented.

THE GOMPERTZ FUNCTION AND TUMOR GROWTH

For tumor growth the Gompertz function can be written as

$$S = S_0 e^{(1-e^{-\alpha t})\beta/\alpha} \tag{1}$$

where S is tumor size at time t, S_0 is the initial tumor size, and α and β are constants. Tumor size may be expressed as weight, volume, or cell number. Figure 1 shows the Gompertz curve for the transplantable hamster tumor, melanotic melanoma No. 1 (15), extrapolated to one cell. Extrapolation of the Gompertz curve outside the experimental range of tumor measurements and the technique for obtaining the parameters will be discussed later.

As time increases the Gompertz function asymptotically approaches a limiting value given by

$$S_\infty = \lim_{t\to\infty}(S_0 e^{(1-e^{-\alpha t})\beta/\alpha}) = S_0 e^{\beta/\alpha}. \tag{2}$$

This quantity, which will be referred to as the "theoretical maximum size" of the tumor, is usually larger than the largest observed tumor size and represents the maximum size the tumor is expected to attain.

Equation 1 is the exact solution to the following pair of differential equations:

$$\begin{aligned} \frac{dS}{dt} &= \lambda S \\ \frac{d\lambda}{dt} &= -\alpha\lambda \end{aligned} \tag{3}$$

where λ is a time-dependent proportionality factor. Integrating equation 3 gives equation 1, where β is the specific growth rate of the tumor at $t = 0$. The growth rate at any time t is given by

$$\lambda = \beta e^{-\alpha t}. \tag{4}$$

Equation 3 describes tumor growth as being, at every instant, exponential but with a simultaneous exponential decrease of the specific growth rate. However, another interpretation (21) of the Gompertz growth function can be obtained by differentiating equation 1. Thus,

$$\frac{dS}{dt} = kS - \theta \tag{5}$$

where $k = \alpha \ln S_\infty$ and $\theta = \alpha S \ln S$. This suggests that k may be identified as an intrinsic growth rate, and θ measures the decrease in actual growth rate as the tumor increases in size. It is reasonable to expect the intrinsic growth rate to be associated with binary fission which results in exponential growth of asynchronously dividing cell populations.

An additional useful property of the Gompertz function is the doubling time of a tumor of size S given by

$$D(S) = \frac{1}{\alpha}\ln\left(1 - \frac{\ln 2}{\ln(S_\infty/S)}\right), \quad S < 1/2\, S_\infty. \tag{6}$$

The doubling time of a hypothetical single-cell tumor may be found by setting S to unity. Therefore,

$$D(1) = \frac{1}{\alpha}\ln\left(1 - \frac{\ln 2}{\ln S_\infty}\right). \tag{7}$$

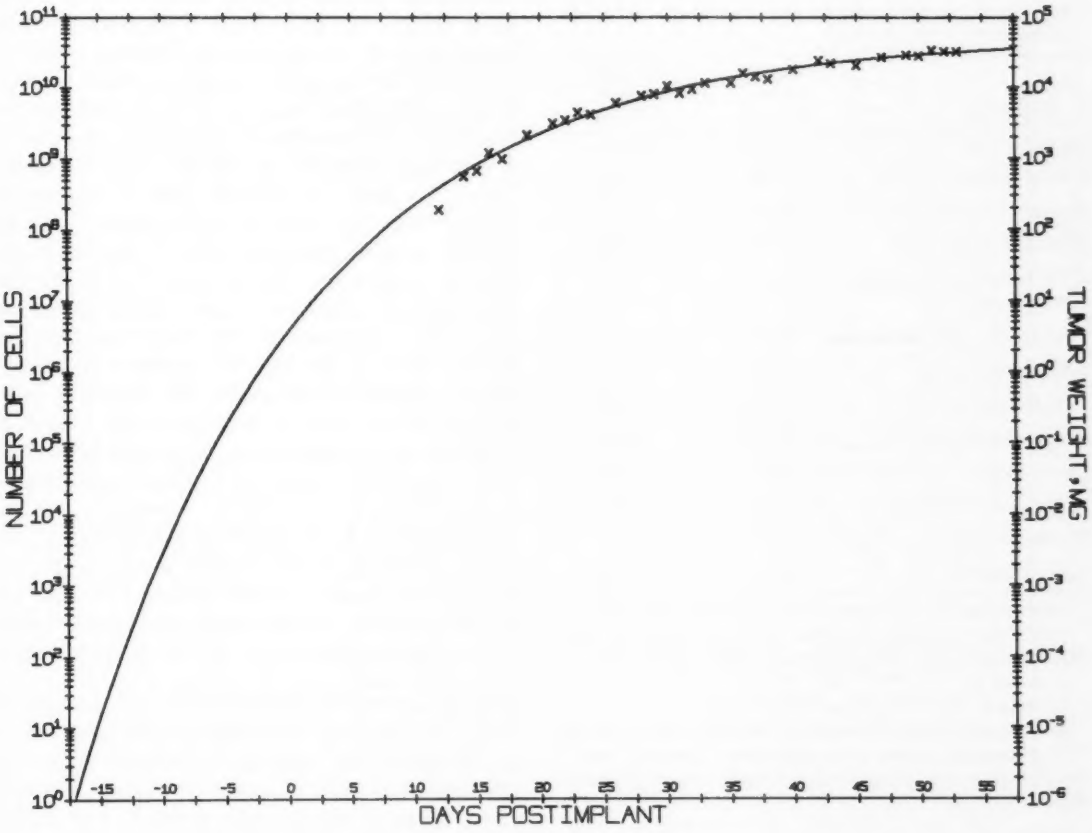

FIGURE 1.—Gompertz growth curve for hamster melanotic melanoma No. 1. The curve was obtained from weighted averages of tumor dimensions. Initially, there were 200 tumor-bearing animals. Gompertz parameters for the computer-drawn curve shown are: $\alpha = 0.0554$ day^{-1}, $\beta = 0.530$ day^{-1}, and $S_0 = 3.82$ mg.

Since $ln\ 2/ln\ S_\infty$ is small, compared to unity, equation 7 reduces approximately to

$$D(1) = \frac{\ln 2}{\alpha \ln S_\infty} = \frac{\ln 2}{k}. \quad (8)$$

It is now seen that the intrinsic growth rate k can be identified with the rate of growth of a hypothetical single-cell tumor. Therefore, $D(1)$ might be expected to correspond to the cell-cycle times determined by the method of labeled mitoses. Table 1 gives a comparison between doubling times computed by equation 7 and cell-cycle times measured by the method of labeled mitoses. It is seen that in general the quantitative agreement is not good, although the discrepancy is not large. However, the predicted single-cell tumor doubling times $D(1)$, although of the right order of magnitude (a few tenths of a day), suggest that extrapolation of the Gompertz curve below the range of palpability may predict tumor sizes that are too small. This will be considered with greater detail in the Discussion.

TREATMENT AND CELL KILL

Theory

If tumor growth curves are to be utilized in estimating the effectiveness of a therapeutic regimen, then some relationship between cells

TABLE 1.—Comparison of hypothetical single-cell tumor doubling times predicted by extrapolation* of Gompertz growth curves and measured cell-cycle times

Tumor	Doubling time (days)	Cell-cycle time† (days)
Sarcoma 180	0.12	0.54‡
DMBA-induced	0.14	0.92‡
Amelanotic melanoma	0.15	—
Adenocarcinoma 755	0.16	0.50‡
AKR lymphoma (sc, 4th generation)	0.17	1.1§
Walker 256 carcinosarcoma	0.18	—
Ehrlich carcinoma	0.24	0.80‡
B16 melanoma	0.26	0.60§
Plasmacytoma No. 1	0.27	0.58‡
Lewis lung	0.35	0.59§
L1210 leukemia (ip)	0.40	0.53‡
Melanotic melanoma No. 1	0.51	0.73‡
C3H mammary	0.69	0.73¶

*Assuming 1 g of tumor is equivalent to 10^9 cells.

†Only the smallest observed value is listed. In general, the cell-cycle time increases with tumor size.

‡*See* ref 15.

§Simpson-Herren L. Unpublished data.

¶Doubling time is computed for the transplanted C3H mammary tumor (15), while the cell-cycle time is for Mendelsohn's (fast line) transplanted C3H mammary tumor (23).

surviving therapy and growth must be found. Such a relationship can be formulated based upon the following assumptions:

a. A Gompertz function can be found which will be valid over the entire growth range (measurable and unmeasurable) of the tumor.

b. Cells affected by treatment are killed instantaneously, or at least within an interval small compared to the period of regrowth.

c. Cells surviving treatment begin to repopulate immediately, producing tumor growth in accordance with the same Gompertz function found for the untreated tumor growth.

Consider now a tumor treated once. Figure 2 illustrates the expectations based on the above assumptions and what is typically observed. Two identical Gompertz curves are shown, separated in time by ϵ units. One represents the growth of an untreated tumor; the other represents the growth of the tumor recurring after treatment. (Since the Gompertz function asymptotically approaches a limiting value independent of the initial boundary value of time, it does not appear visually obvious that the two Gompertz curves remain separated in time by a constant amount as tumor size increases. This fact may be verified, but it should be noticed that the vertical displacement does diminish as time increases.) Let T represent the time of treatment. The broken line illustrates the typically observed tumor mass behavior. The tumor increases in size until treatment, at which time it diminishes perhaps to a size below palpable dimensions. The negative slope of the broken line is the combined result of removal of the dead tumor mass and growth of surviving tumor mass. Provided the treatment is noncurative, the tumor eventually recurs. The dotted line illustrates the behavior of the viable fraction of the tumor (assumption *b*). The intersection of the dotted line with curve R represents the viable tumor size immediately following therapy, and it is this surviving portion that continues to grow. The surviving portion of the tumor is also represented by $S(T\text{-}\epsilon)$ on the untreated tumor Gompertz curve. To estimate the fraction of tumor surviving therapy, it is only necessary to have the Gompertz curve of the untreated tumor and an estimate of ϵ. Since the displacement of the two curves is presumed constant, ϵ may be determined at any convenient point. The fraction of the tumor surviving treatment may be formulated as

$$F = \frac{S(T-\epsilon)}{S(T)}. \qquad (9)$$

Estimating ϵ From Experimental Data

Provided that the Gompertz growth curves of the control and treated tumors are identical as assumed, ϵ may be determined at any point along the curve. However, practical considerations limit the desirable points. It is obvious that the estimate must be made at a point when the tumor is of measurable size. In addition, it would be desirable to minimize the delay in evaluation. Thus, it seems most convenient to

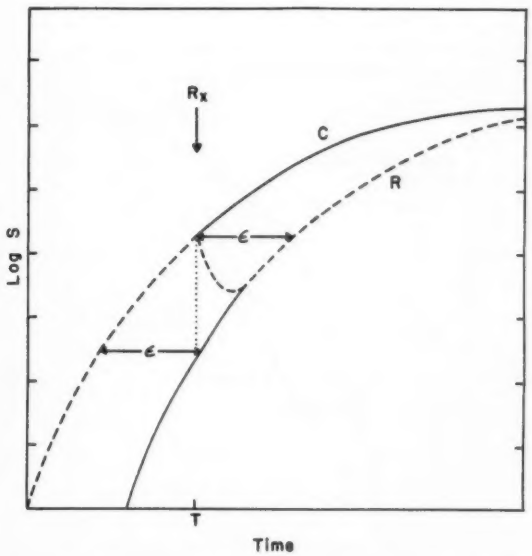

FIGURE 2.—Schematic representation of tumor growth and effects of a single noncurative treatment. T = time of treatment; C = growth curve of untreated tumors; R = growth curve of treated recurring tumors (same Gompertz function as curve "C"); ϵ = displacement of "R" with respect to "C."

choose ϵ as the displacement between the two growth curves at a point as near as possible to the tumor size at initial treatment. This value of ϵ may be compared to the displacement intervals at other points along the curve for verification. If ϵ does not remain reasonably constant, then the growth curve and the recurring curve are not identical Gompertz functions and the applicability of using this procedure for determining cell kill is questionable. However, in such cases a range for ϵ may still provide useful information.

Figures 3 and 4 illustrate typical experiments and the estimation of ϵ. When plasmacytoma No. 1 was treated with 25 mg/kg of cyclophosphamide [8] on Day 8 (fig 3) there followed a period during which the tumor size increased before regression occurred (20). ϵ was taken as the interval between the control curve and the recurring curve for the tumor mass at the time of treatment. When melanotic melanoma

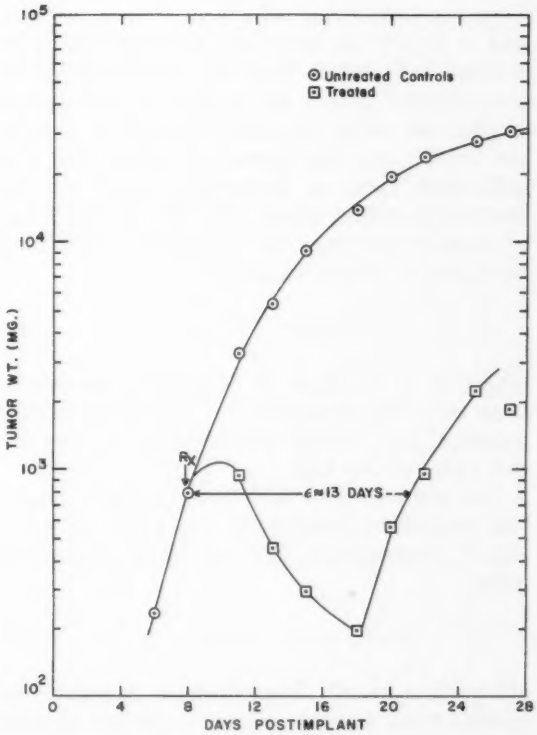

FIGURE 3.—Estimation of ϵ for hamster plasmacytoma No. 1 treated with 25 mg/kg of cyclophosphamide on Day 8. The computer-fitted Gompertz curve of the untreated controls is shown ($\alpha = 0.137$ day^{-1}, $\beta = 1.60$ day^{-1}, $S_0 = 0.333$ mg). The curve for the treated tumors is hand-drawn.

No. 1 was treated with uredepa [9] (20 mg/kg on Day 14), the tumor increased in size for about 2 days, followed by an interval of constant size.[10] ϵ was taken as the interval between the control and recurring curves at a point above the tumor size at treatment.

Estimation of Tumor Cell Kill

The Gompertz parameters α and β may be determined by taking the natural logarithm of equation 1, giving

$$u = a_0 + a_1 v \qquad (10)$$

[8] Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate.

[9] Uredepa: NSC-37095; CAS reg. No. 302-49-8; carbamic acid, [bis(1-aziridinyl)phosphinyl]-, ethyl ester; AB-100.

[10] Griswold DP, Jr. Unpublished data.

where $u = ln\ S$, $a_0 = ln\ S_0 + \beta/\alpha$, $a_1 = -\beta/\alpha$, and $v = e^{-\alpha t}$. A computer program has been written (15) which finds the best a_0 and a_1 by a systematic search on α. When a_0 and a_1 are known, the values of α and β follow. If α and β are known for the untreated tumor and ϵ is estimated from a therapeutic trial by the method described above, then the log kill [11] (y) of tumor cells may be estimated. Combining equations 1, 9, and 11 gives

$$y = \frac{\beta}{\alpha \ln 10} e^{-\alpha T}(e^{\alpha\epsilon} - 1) \qquad (12)$$

where T is the time of treatment relative to time zero. Direct substitution of the numerical values of α, β, ϵ, and T into equation 12 provides the value of log kill.

Log kill may also be determined by a graphical procedure. Taking the logarithm of equation 1, rearranging, and taking the logarithm gives

$$\log \log (S_\infty/S) = \log (\beta/b\alpha) - \frac{\alpha}{b}t \qquad (13)$$

where $b = ln\ 10$. Therefore, a straight line results when log log (S_∞/S) is plotted against t. Since the slope of the straight line is the negative of α, this transformation may be used to estimate the Gompertz parameters (18,22). It is first necessary to guess at S_∞ and then plot the growth data according to equation 13. If the estimate of S_∞ is high, the plotted points will be displaced upward from a straight line at larger values of t; if the estimated S_∞ is low, the plotted points will exhibit a downward curvature. Based on the behavior of the plotted points, the estimate of S_∞ may be adjusted and the data replotted. Examples using exact values of S_∞ are shown in figures 5 and 6 which utilize the same data seen in figures 3 and 4 respectively.

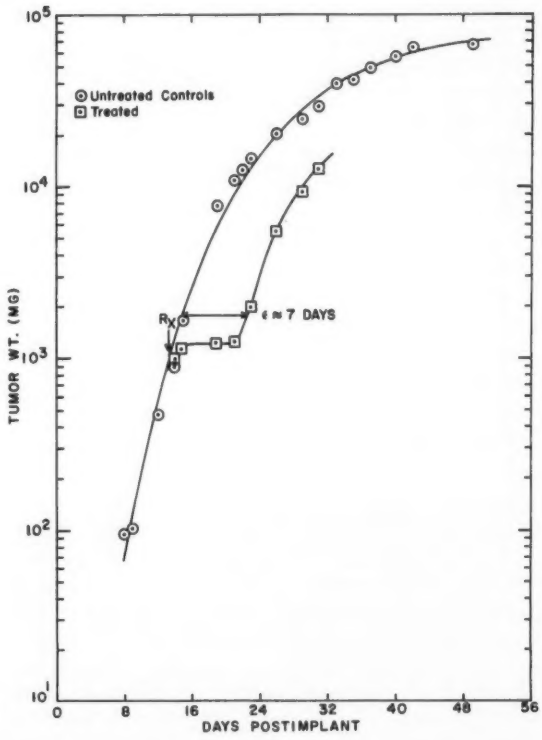

FIGURE 4.—Estimation of ϵ for hamster melanotic melanoma No. 1 treated with 20 mg/kg of uredepa on Day 14. The computer-fitted Gompertz growth curve of the untreated controls is shown ($\alpha = 0.0881$ day^{-1}, $\beta = 1.28$ day^{-1}, $S_0 = 0.0442$ mg). The curve for the treated tumors is hand-drawn.

To demonstrate that log kill may be estimated from plots as shown in figures 5 and 6, let

$$A = \log (S_\infty/S(T)) \qquad (14)$$

and

$$B = \log (S_\infty/S(T-\epsilon)). \qquad (15)$$

[11] The term "log kill" is defined formally as

$$\text{log kill} \equiv y = -\log(F) \qquad (11)$$

where F is the fraction of tumor surviving treatment. It provides the means of accommodating a wide dynamic range of the variable (in this instance, F) in a dimensionless quantity of convenient magnitude. For example, if the fraction surviving is 0.1, then log kill = 1.0, or if the fraction surviving is 0.0000000001, then log kill = 10.0. Not only is a log kill easily converted to the fraction surviving, but it also represents the number of log units by which the tumor is reduced. If, for instance, a tumor size of 10^{10} cells is subjected to a treatment which results in a 4.5 log kill, then the number of cells remaining is $10^{5.5}$.

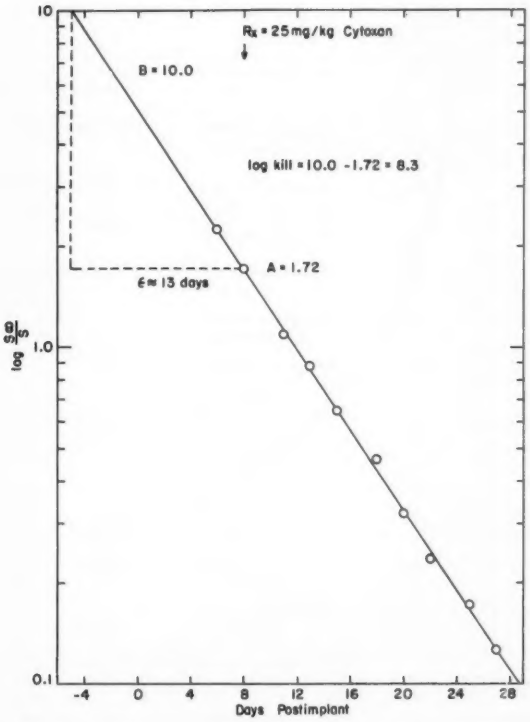

FIGURE 5.—Transformed Gompertz curve for the group of untreated animals with hamster plasmacytoma No. 1 shown in fig 3. A separate group of animals with established tumors was treated on Day 8 with 25 mg/kg of cyclophosphamide. It required about 13 days for the treated tumors to return to their size at treatment.

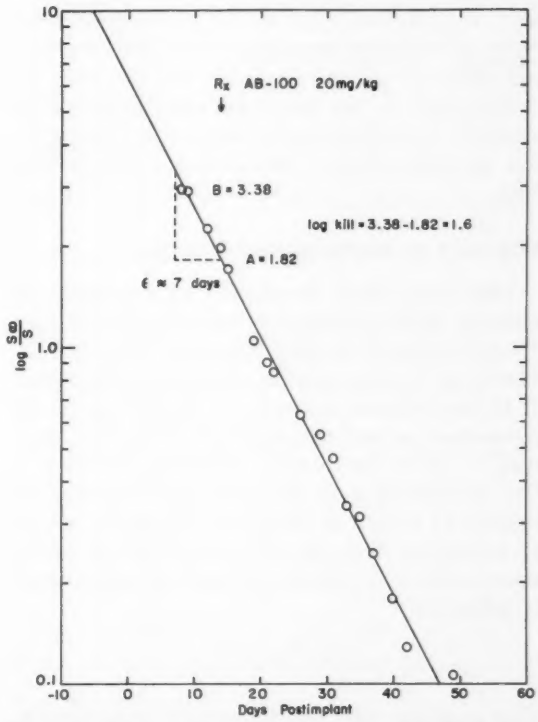

FIGURE 6.—Transformed growth curve for the group of untreated animals with melanotic melanoma No. 1 shown in fig 4. A separate group of animals with established tumors was treated on Day 14 with 20 mg/kg of uredepa. It required about 7 days for the treated tumors to return to their size at treatment.

Since the plots are semilogarithmic, the numerical value of $\{S_\infty/S(t)\}$ for any t is given directly on the logarithmic ordinate. Therefore,

$$B - A = -\log \frac{S(T-\epsilon)}{S(T)} = y \equiv \log \text{kill}. \qquad (16)$$

Error Analysis

It is of interest to ascertain the effect that error in the estimate of ϵ has upon the evaluation of log kill. The relative error in y, or log kill, is given approximately by

$$\frac{\Delta y}{y} = \frac{1}{y}\frac{\delta y}{\delta \epsilon}\Delta\epsilon. \qquad (17)$$

By differentiating equation 12 with respect to ϵ, and on substituting the expression for the derivative and for y into equation 17, the relative error becomes

$$\frac{\Delta y}{y} = \frac{\alpha}{1 - e^{-\alpha\epsilon}}\Delta\epsilon. \qquad (18)$$

Thus, the relative error in log kill is dependent only on the magnitude of α and the time required for the treated tumors to recur. Figure 7 is a graph of the relative error per unit of error in ϵ for several values of α. It is to be noted that the relative error decreases and approaches α as ϵ becomes large. For small cell kill and consequently small ϵ, the relative error is large, approaching infinity as ϵ approaches zero. Thus, the largest uncertainty in cell kill due to error in ϵ occurs when the cell kill is small. Typically, α is usually between 0.02 and

0.25 day^{-1}. For values of ϵ between 7 and 21 days, the relative error in log kill for each day error in ϵ will be expected to be between 5% and 20%.

The error in the graphical procedure would probably be a little larger due to the inaccuracies in estimating A and B from the plotted data.

Extension to Multiple-Dose Therapy

The theory may be applied to multiple-dose therapy. Suppose that treatment begins on Day T and continues at intervals until Day $T + d$. The duration of treatment extends over a period d. If the interval between Gompertz curves of the untreated and treated tumors is given as ϵ, then $\epsilon - d$ is the interval between the time of last treatment and the time for the treated tumors to return to their size at the initiation of treatment. The log kill at the end of treatment relative to the time treatment was started is given by

$$y = \frac{\beta}{\alpha \ln 10} e^{-\alpha T}(e^{-\alpha(\epsilon-d)}-1). \quad (18)$$

This equation may be compared to equation 12, and may be considered to describe both single-dose therapy ($d = 0$) and multiple-dose therapy ($d > 0$).

COMPARISON OF THEORY TO EXPERIMENT

Tumors were implanted subcutaneously as trocar fragments from donor animals. When the implanted fragments grew to palpable dimensions, caliper-measured diameters were converted to approximate mass assuming unit density by using the equation:

$$\text{Volume} = 1/2 \times \text{length} \times (\text{width})^2. \quad (19)$$

The drug was administered to the treated group of animals by intraperitoneal injection. Bioassay (20,24) was performed by preparing a series of tumor breis (usually 20%, 2%, and 0.2% wt/vol) and injecting intracerebrally 0.05 ml of each dilution into each of ten recipient animals. The median lifespan of the recipient animals was compared to a standard curve from which the number of cells in the tissue brei could be calculated. At several intervals after treatment, bioassays were performed on treated and control animals, and the minimum bioassay result was usually obtained 24–48 hours after treatment. The fraction of cells surviving therapy was found by taking the ratio:

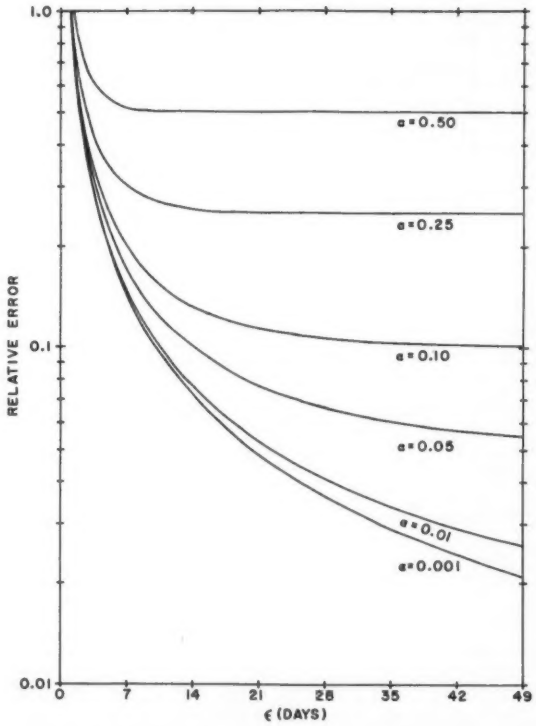

FIGURE 7.—Relative error in "log kill" per unit of error in ϵ; the time required for the treated tumors to return to their size at treatment.

$$f_s = \frac{\text{Bioassay result after treatment}}{\text{Bioassay result of control at time of treatment}}. \quad (20)$$

The Gompertz parameters were optimized (15) to the tumor weights of the untreated controls; ϵ was estimated and the fraction surviving was calculated using equation 9 as previously described. A comparison of log kill determined from bioassay and Gompertz growth curves is given in table 2.

DISCUSSION

Equation 6 gives the doubling time for any

TABLE 2.—Comparison of solid tumor cell kill estimated from bioassay and Gompertz growth curves

Tumor	Treatment*	Dose (mg/kg)	Time after treatment bioassay performed	Gompertz parameters ($days^{-1}$)		ε (days)	Log kill	
				α	β		Gompertz†	Bioassay‡
Walker 256 carcinosarcoma (sc)	Cyclophosphamide (Day 8)	60	4 hrs	0.175	2.33	7	3.4	3.4
			24 hrs					4.0
Ehrlich carcinoma	Cyclophosphamide (Day 10)	120	24 hrs	0.0683	0.462	18	3.6	3.9
Melanotic melanoma No. 1	Cyclophosphamide (Day 14)	90	4 hrs	0.0702	0.849	2	0.3	0.3
			48 hrs					0.8
	Uredepa (Day 14)	20	5 days	0.0881	1.28	7	1.6§	1.9
Plasmacytoma No. 1	Cyclophosphamide (Day 8)	25	4 hrs	0.137	1.60	13	8.4¶	>5.0
	Cyclophosphamide (Day 9)	20	24 hrs	0.129	1.37	12	5.3	4.2
	Cyclophosphamide (Day 9)	25	4 days	0.127	1.44	12–13	~6.1	3.4
B16 melanoma	CCNU (Day 8)	50	48 hrs	0.083	0.613	13	3.2	4.4**
		33				7	1.3	4.2**
	Methyl-CCNU (Day 8)	36	48 hrs	0.083	0.613	6–7	1.2	5.1**
		24				4–6	0.8	4.9
	Methyl-CCNU (Day 11)	36	4 hrs	0.102	0.735	13–15	~3.3	2.3
			7 days					3.0
	BCNU (Day 8)	39	48 hrs	0.083	0.613	4–6	~0.8	2.0
		26				2–4	~0.5	0.7
	Cyclophosphamide (Day 10)	200	4 hrs	0.0663	0.425	5–10	~1.0	0.8

*All drugs were administered intraperitoneally.
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.
†From equations 9 and 12, using "best fit" Gompertz curve of untreated control tumors and estimated ε.
‡From equations 20 and 11.
§*See* fig 4.
¶*See* fig 3.
**Bioassay at only one dilution level; not replicated.

size tumor that is less than half the maximum theoretical size. This represents the time along the Gompertz curve required for the tumor to double in size, ie, to change from S to $2S$. Therefore, once a tumor has reached size $\frac{1}{2}S_\infty$ it can no longer double. Equation 4 gives the specific growth rate of the tumor at any time (or for a corresponding tumor size). It represents a tangent to the Gompertz curve at time t. Thus,

$$T_d = \frac{\ln 2}{\lambda} \qquad (21)$$

represents an "instantaneous" doubling time of the tumor at the time t for which λ is given by equation 4. It gives the doubling time of the tumor if it would continue to grow with the rate at time t. The value of T_d is more representative of the growth state of the tumor at time t than is $D(S)$.

Most Gompertz-fitted tumor growth curves are obtained from tumor dimensions over the palpable range of tumor size. In some instances, such as for transplantable experimental leukemias and some solid tumors, the number of viable cells in small inocula may be estimated indirectly from bioassay or lifespan data. However, for those cases where direct or indirect measurements are not possible, the extrapolation of the Gompertz-fitted growth curve may provide additional information about the growth of very small tumors. Caution in interpretation must always be exercised when extrapolating a model far beyond the range in which the parameters of that model were determined. For example, the extrapolations in figure 1 and table 1 are predicated on the assumption that the Gompertz model is valid from one cell to death of the host. This assumption has

not been verified by a direct experimental test for solid tumors but appears reasonably correct for L1210 leukemia (15). The magnitude of the single-cell doubling times predicted by extrapolation (table 1) tends to be less than the minimum observed cell-cycle time. Extrapolation of the Gompertz curve will probably be accurate to a point where the "instantaneous" doubling time is approximately equal to the minimum observed cell-cycle time, but below this point the extrapolation will predict growth rates that are too high and thus tumor sizes that are too small. Also influencing the quantitative results of the extrapolation is the assumption of the equivalence of 10^9 tumor cells and 1 g of tumor tissue, and the fact that the estimates of the Gompertz parameters, and consequently $D(1)$, are subject to the growth data available. There is variation in the magnitude of the Gompertz parameters for the same tumor type among individual animals and among groups from which average tumor sizes are utilized.

The theory for using Gompertz growth curves for the purpose of estimating cell kill is based upon three explicit assumptions. Generally, a Gompertz function can be found that will adequately fit experimental tumor growth data. The same function is then assumed to be valid over the entire growth range of the tumor. Exponential growth ($\alpha \rightarrow 0$) is a special case of the Gompertz function. The assumption of instantaneous cell death as a result of treatment is not restrictive. For chemotherapeutic treatment, cells are probably killed over an interval at least equal to the duration of effective drug concentration. It is only necessary that the cumulative effects causing the delay in growth or delay in reappearance of the tumor mimic instantaneous cell death. The third assumption, though required for the mathematical development, is reasonable.

Implicit within the theoretical development is that the change in tumor size or the corresponding displacement in time of the recurring tumor resulting from treatment is somehow related to the effect that the treatment has upon the number of viable cells present in the tumor. No attempt is made to define this relationship. However, even with this undefined relationship and the three explicit assumptions, the data in table 2 suggest that frequently, though not uniformly, a good correspondence exists between tumor cell kill as determined by bioassay and tumor cell kill as determined from an estimate of the displacement (ϵ) of the treated and untreated Gompertz curves. Both methods are subject to error. The bioassay has only order-of-magnitude accuracy (20). Log kill from growth curves is dependent upon "fitting" the Gompertz function to growth data, the precision of the estimate of ϵ, and the degree to which the theory is suitable.

The graphical procedure, though less precise for estimating log kill, has some meritorious features. First, estimates of the Gompertz parameters are not required; thus the necessity of developing a computer program is avoided. If needed, these parameters may be obtained from the graphical procedure but with less precision than from a numerical procedure. The graphical procedure requires an initial estimate of S_∞, but this estimate can usually be obtained from a semilogarithmic plot of the control tumor growth data. Subsequent adjustments to this estimate can be made based on the appearance of the curve when $log\ log\ S_\infty/S$ is plotted against time. The graphical procedure would not be the method of choice for obtaining Gompertz parameters for a large number of growth curves. Second, the log kill may be read directly from the logarithmic axis, once an estimate of ϵ has been found. Third, for a group of therapeutic trials which are compared to the same control, the graphical procedure provides an easy and convenient method for evaluating the treatment effectiveness.

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Determination of 5-Fluorouracil (NSC-19893) Plasma Levels in Rats and Man by Isotope Dilution-Mass Fragmentography [1,2,3,4]

C. Finn [5,6] and W. Sadée [5,6,7,8]

SUMMARY

A highly sensitive and specific rapid assay for 5-fluorouracil (5-FU) in plasma samples at concentrations as low as 1 ng/ml was developed using mass fragmentography. Highly enriched C^{14}-5-FU was employed as an internal standard. A two-compartmental open model for the disposition of free 5-FU in plasma is proposed. The β-phase of 5-FU plasma elimination describes the pool size of anabolic metabolites, assuming this model is correct. Injections of 5-FU at 15, 40, and 90 mg/kg in rats resulted in an α-phase of elimination from plasma with a half-life of 10–18 minutes. The β-phase could only be measured after the injection of 90 mg/kg of 5-FU and amounted to about 20 hours in two rats. Higher doses resulted in an unproportional increase in 5-FU plasma concentrations in the β-phase, thus establishing nonlinear kinetics, presumably of the degradative metabolism. Plasma concentrations of 5-FU in man were measured after oral and intravenous administration of 15 mg/kg of 5-FU. Plasma elimination half-lives after intravenous doses were 12 minutes and dropped below measurable levels 2 hours after administration in three patients. After oral administration, peak plasma levels of 5-FU varied between 0.8 and 60 μg/ml of 5-FU in three patients after 10 minutes to 2 hours. Two of these patients showed rapid absorption and a measurable β-phase of 5-FU plasma elimination possibly indicating a larger anabolic pool size of 5-FU-derived nucleosides and nucleotides when compared to patients receiving intravenous doses. This study may provide a pharmacokinetic rationale to monitor patients receiving 5-FU treatment.

[Cancer Chemother Rep 59:279–286, 1975]

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[2] Supported by Public Health Service grant CA-05186 from the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health, Education, and Welfare. Mass spectral analysis was supported in part by Public Health Service grants CA-14089 from the NCI and GM-16496 from the National Institute of General Medicine, NIH.

[3] 5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU.

[4] This study was performed by the Western Cancer Study Group.

[5] Clinical Pharmacology and Pharmacokinetics Section, Schools of Medicine and Pharmacy, University of Southern California, Los Angeles.

[6] *Present address*: School of Pharmacy, University of California, San Francisco.

[7] We gratefully acknowledge contributions to this study by Drs. Richard Brown and Howard Darvey.

[8] Detailed information on the accelerating voltage alternator (AVA) and *reprint requests to*: W. Sadée, PhD, School of Pharmacy, University of California, San Francisco, Calif 94143.

The antineoplastic agent 5-fluorouracil (5-FU) distributes rapidly throughout the body tissues (1,2) and is mainly eliminated by urinary excretion and metabolism (3). The first step of the degradative metabolism leading to pharmacologically inactive metabolites represents a reduction to dihydro-5-FU which appeared to be an easily saturable hepatic pathway (4). A fraction of 5-FU enters the anabolic pool of 5-FU-derived nucleosides and nucleotides which contains the major pharmacologically active principle, namely, 5-fluoro-2′-deoxyuridine-5′-monophosphate (5-FdUMP) (5–7). Incorporation of 5-FU into RNA (6) can be assumed to play a negligible role in the overall pharmacokinetics of 5-FU. Elimination of metabolites from the anabolic pool was reported to occur predominantly via 5-FU and its further metabolic degradation (2). We thus propose a pharmacokinetic model of the disposition of 5-FU as a rationale for our experimental approach (fig 1).

The major exposure to the active metabolite, 5-FdUMP, in mice occurred 24–72 hours after the administration of 5-FU (8) although 5-FU plasma levels were reported to fall below measurable limits within a few hours after administration of 5-FU in man (9). Assuming that the proposed model (fig 1) is correct, one has to expect a biexponential decay curve of 5-FU plasma levels. The terminal log-linear phase of the elimination of 5-FU (β-phase) should then provide an indirect relative measure of the anabolic pool size and thus the amount of active metabolite formed.

Prerequisite for this approach to 5-FU kinetics is a highly sensitive and specific assay of 5-FU suitable for plasma samples. We selected gas chromatography-mass fragmentography as the analytical tool in this study. Monitoring single ions by mass spectrometry results in a large increase of specificity and sensitivity over conventional flame ionization detection (10) which was previously employed to measure 5-FU in biologic samples (11,12). Accurate quantitation can be achieved by means of isotope dilution techniques, which we have used in previous studies (13). Since no 5-FU labeled with stable isotopes was available to us, highly enriched C^{14}-5-FU (< 80% C^{14}) was

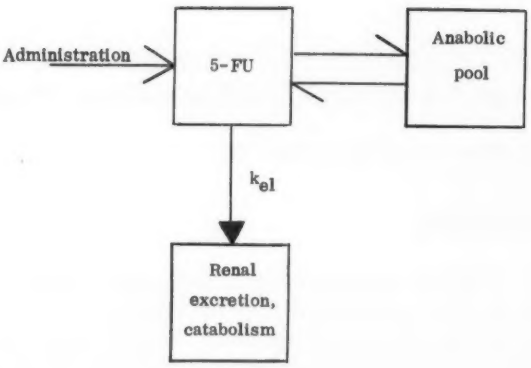

FIGURE 1.—Proposed pharmacokinetic model of the disposition of 5-FU.

employed in this study. Contamination of the mass spectrometer by C^{14} radioactivity was negligible because amounts of internal standards injected on column were kept below 200 picograms (pg) of 5-FU representing less than 200 dpm/sample.

MATERIALS AND METHODS

A gas chromatograph-mass spectrometer accelerating voltage alternator (AVA), which was a Varian CH-7 coupled via a two-stage Watson-Biemann separator to a Varian model 2740 gas chromatograph, was used. The mass spectrometer was modified by the addition of a highly stable accelerating voltage supply ("Fluke 415B") and a six-channel AVA of our own design. This AVA has a mass range of 50% and a channel dwell time variable from 100 milliseconds to 10 seconds. The accelerating voltage was monitored by a five-digit meter to a resolution of one part in 30,000. Since the CH-7 has a water-cooled magnet, heating effects are minimized; after a stabilization period of 5 minutes, magnet drift at m/e 300 is less than 0.05 atomic mass unit/hour.

Gas Chromatograph-Mass Spectrometer AVA Conditions

A 6-foot by ¼-inch glass column packed with 1% wt/wt Pentasil 350 (Applied Science) coated on Chromosorb G 100/120 mesh was used. The injector, column, separator, and source temperatures were 150°, 100°, 180°, and

180° C, respectively. Accelerating voltages were 3000.0 and 2977.0 volts for masses 259 and 261, emission current was 300 microamperes, multiplier voltage was 1.75 kilovolts, channel dwell time was 200 milliseconds, filter was 10 Hertz, chart speed was 10 cm/minute, and resolution was 500.

Internal Standard

Because of the unavailability of stable isotope-labeled 5-FU, highly enriched C^{14}-5-FU was used for the internal standard in the assay. The C^{14}-5-FU used had a quoted specific activity of 52 millicurie/mmol at position C-2 (Mallinckrodt, NY) and a measured isotopic enrichment of 81% C^{14}.

Liquid Scintillation Apparatus

All samples were counted in 10 ml of Aquasol (New England Nuclear Corp.) in polyethylene vials to 10,000 counts or 20 minutes in a Unilux II counter (Nuclear Chicago) with a C^{14} efficiency of 75%–85%, and counts were corrected by the channels-ratio method.

5-FU Assay Procedure

A total of 0.5 ml of plasma was placed in a ground glass stoppered tube. Internal standard solution (100 μl) containing 3000 dpm (about 3 ng) of 5-FU was added along with 0.5 ml of a saturated solution of ammonium sulfate. Due to the small amount of internal standard added (3 ng), aliquots of plasma containing from 0.5 to 20 ng were used and the volume was adjusted to 0.5 ml with distilled water. The aqueous layer was extracted once with 7 ml of 20% *n*-propanol in diethyl ether. The organic phase was transferred to a disposable test tube and blown dry with nitrogen in a heating block. The residue was transferred to a 1-ml "Reacti-Vial" (Pierce Chemical Co.) with a single wash of 0.2 ml CH_2Cl_2. This plasma extraction procedure gave an overall yield of between 50% and 70% for all samples. The CH_2Cl_2 was blown off with nitrogen and 10 μl of *N,O*-(bis)-(trimethylsilyl)-trifluoroacetamide (BSTFA) and pyridine in a ratio of 1:4 was added. The "Reacti-Vial" was heated for 10 minutes at 60°C in a heating block. Usually, 1–2 μl was injected in the gas chromatograph-mass spectrometer. The retention time of the disilylated derivative was 1.5 minutes.

The ditrimethylsilyl derivative of 5-FU gives a parent ion at m/e 274 with 15% intensity relative to the base peak at m/e 73. The M^+-15 peak, however, yields an intensity of 70% relative to the base peak, and accounts for approximately 10% of total ionization. Single ion monitoring of m/e 259 resulted in a mass chromatogram peak for as little as 5 pg of a pure derivatized sample of 5-FU injected on column with a signal to noise ratio of 1. Thus, the peak M^+-15 m/e 259 was chosen for the assay; for the internal standard labeled with one C^{14} in position C-2, the mass m/e 261 was monitored. By using the AVA method, peak height ratios of masses at m/e 259/261 corresponding to M^+-CH_3 fragment ions of 5-FU and C^{14}-5-FU were determined. Standard curves were constructed from plasma samples containing from 1 to 80 ng/ml of 5-FU. Because the internal standard was labeled only to the extent of 81%, its contribution to m/e 259 was 19% leading to a standard curve which did not pass through the origin. 5-FU (1 ng/ml) could be detected above blank for most samples although one patient's plasma had large interfering ions at both m/e 259 and 274, allowing measurements only down to 100 ng/ml; this patient was not included in this study. For a standard of 10 ng/ml extracted from plasma the standard deviation was 6%.

Specificity of the Assay Procedure

Blank samples should be run with each experimental subject to determine the respective obtainable level of sensitivity. Possible fluctuations of plasma blank readings by endogenous uracil and thymine pool size changes as a result of 5-FU treatment were ruled out since the 5-FU plasma determinations were not affected by adding uracil and thymine at various concentrations.

Further evidence for specificity was obtained by monitoring multiple ions of the gas chromatograph eluate. 5-FU (50 mg/kg) was injected intravenously (iv) into one rat and plasma samples were obtained after 30 and 60 minutes. The samples were run through the

assay procedure without adding C^{14}-5-FU as a standard and the ions at m/e 259, 261, 274, and 276 were monitored corresponding to M^+-CH_3, M^+-CH_3+2, M^+, and M^++2, respectively. The calculated contribution of naturally occurring stable isotopes of silylated 5-FU, ie, C-13, Si-29, Si-30, and O-18, to the M^++2 ion was 8.3% of the M^+ ion. The measured ion intensities of a pure sample of 5-FU were 8.1% for M^++2 relative to M^+ and 7.3% for M^+-CH_3+2 relative to M^+-CH_3. These ion ratios were not changed in the 5-FU samples extracted from rat plasma within statistical error (8% for M^++2/M^+ and 7% for M^+-CH_3+2/M^+-CH_3). Thus, catabolic 5-FU metabolites (eg, dihydro-5-FU) do not interfere with the assay.

However, anabolic 5-FU-derived nucleosides and nucleotides can interfere by cleavage of the sugar-base link during the assay procedure. The silylation method using BSTFA and pyridine (1:4) resulted in partial cleavage of 5-fluoro-2′-deoxyuridine (5-FUdR) and 5-FdUMP to give disilylated 5-FU with a 2%–4% yield. Both 5-FUdR and 5-FdUMP were found to be chromatographically pure (< 0.5% 5-FU contamination). The plasma extraction step of the 5-FU assay procedure eliminated the nucleotide 5-FdUMP as an assay-interfering factor but not 5-FUdR which was extractable to the same extent as 5-FU. Therefore, one rat was injected iv with 90 mg/kg of 5-FU and a plasma specimen was obtained after 24 hours. Two identical plasma samples (0.5 ml each) of this specimen were assayed for 5-FU content using C^{14}-5-FU as an internal standard. One of the samples was subjected to a column chromatography step (Sephadex LH-20 [Pharmacia Fine Chemicals, NJ], CH_2Cl_2: CH_3OH = 98:2) which separates 5-FU and 5-FUdR. No difference in the 5-FU reading (30 ng/ml) was found between the two samples, indicating that the nucleoside 5-FUdR as a metabolite of 5-FU did not interfere with the assay.

Patient Protocols

Blood samples were collected as follows. An iv drip was set up using normal saline. The iv drip rate was just fast enough to keep the blood from coagulating around the needle. A small amount of blood was drawn out prior to sampling to avoid any dilutional problems. Three patients received 15 mg/kg of 5-FU iv in the opposite arm. Three different patients received 15 mg/kg of 5-FU orally in aqueous solution which was prepared by diluting iv 5-FU solution with water to about 100 ml. All oral doses were given on an empty stomach in the morning. Breakfast was allowed 1 hour after 5-FU administration. Blood specimens (approximately 5 ml) were collected in test tubes using powdered ethylenediaminetetraacetic acid as the anticoagulate (B-D Vacutainer No. 3204Q). The blood was centrifuged at 2000 rpm for 10 minutes. The plasma was removed and frozen for subsequent analysis. Patients of both sexes selected for this study were between 44 and 73 years old with various neoplastic diseases for which 5-FU was part of their medical treatment (*see* table 1).

Rat Protocols

Female Sprague-Dawley rats weighing 250–300 g were used. Left side carotid cannulas were inserted using PE-20 tubing; the cannulas were flushed with saline after withdrawing blood to prevent clotting. Iv doses of 15, 40, and 90 mg/kg of 5-FU for one rat at each dosage level were dissolved in distilled water made alkaline to a pH level of 10 with NaOH and C^{14}-5-FU was added sufficient to give about 1000–2000 dpm/μg of the dose (1–10 microcurie/animal). Blood samples taken throughout the experiment for up to 24 hours after administration were spun in a Beckman microcentrifuge and the plasma was frozen for later analysis. Two additional rats received iv doses of 90 mg/kg of 5-FU and the plasma samples were obtained between 23 and 48 hours after administration.

RESULTS

Assay Procedure

The described assay procedure for 5-FU plasma concentrations is specific in the presence of 5-FU metabolites and sensitive to about 1 ng/ml. Previously employed assay procedures lack the sensitivity or specificity of this assay. Its preliminary application to pharmacokinetic studies of 5-FU is shown below.

TABLE 1.—Patient data

Patient No.	Age (yrs)	Sex	Route of administration	Diagnosis	Concurrent medication
1	49	M	Iv	Carcinoma of pancreas with metastases to liver	Talwin, chloral hydrate
2	53	F	Iv	Metastatic adenocarcinoma to liver; primary tumor unknown	Tylenol, chloral hydrate
3	73	F	Iv	Adenocarcinoma of colon with metastases to liver	Tylenol, chloral hydrate
4	65	M	Oral	Adenocarcinoma of pancreas with metastases to liver and bone	Chloral hydrate, Dilaudid
5	44	M	Oral	Adenocarcinoma of colon with metastases to liver	Mineral oil, folic acid, codeine
6	61	F	Oral	Adenocarcinoma of colon with metastases to liver	Tylenol, chloral hydrate

Plasma Levels of 5-FU in Rats After Iv Administration

The plasma concentration-time curves of 5-FU and total C^{14}-radioactivity are depicted in figures 2 and 3 after injections of 5-FU at 15, 40, and 90 mg/kg. C^{14}-radioactivity rapidly declined in the first 2 hours after administration and then leveled off due to formation of the 5-FU metabolites. Half-lives in the α-phase of elimination of 5-FU from plasma measured by mass fragmentography were 10 minutes after administration of 15 and 40 mg/kg of 5-FU and 18 minutes after administration of 90 mg/kg of 5-FU (fig 3). Zero-time plasma concentrations of 5-FU were obtained by extrapolation and amounted to 32, 60, and 140 μg/ml representing an initial volume of distribution of 47%, 67%, and 64% of body weight after injection of 15, 40, and 90 mg/kg, respectively.

The initial 5-FU plasma concentration-time curve was log-linear over about 10 half-lives and then started to level off after administration of 15 and 40 mg/kg, respectively. The terminal log-linear phase of plasma elimination (β-phase) could not be measured at these dose levels due to assay limitations (fig 3).

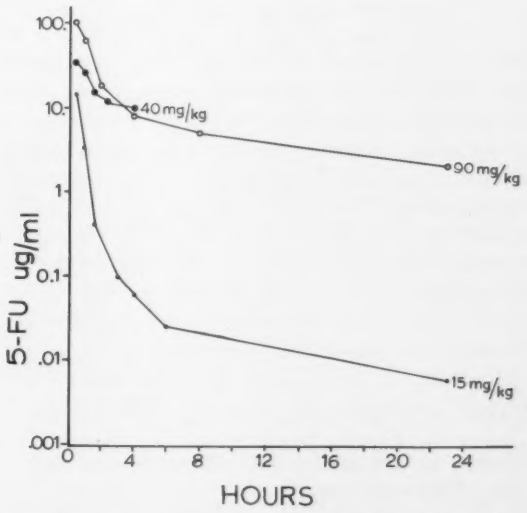

FIGURE 2.—C^{14} plasma activity expressed in 5-FU (μg) equivalents in rats after iv doses of 15, 40, and 90 mg/kg of 5-FU. One rat was used for each dosage level.

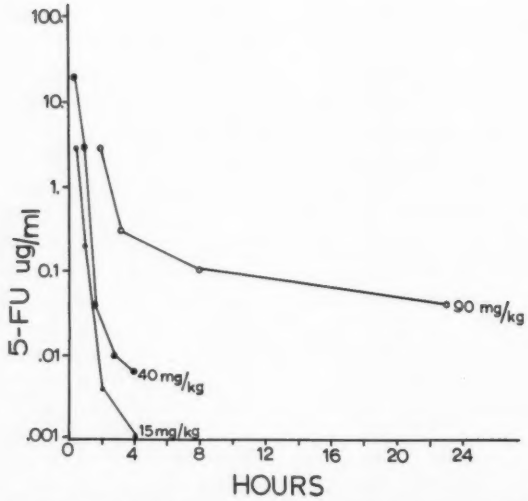

FIGURE 3.—5-FU plasma concentrations by mass fragmentography in the same rat experiments as in fig 2 after iv doses of 15, 40, and 90 mg/kg of 5-FU.

At a dose of 90 mg/kg of 5-FU, plasma levels started to level off into the β-phase much earlier and were observed over 23 hours in one rat (fig 3). Two additional rats were injected with 90 mg/kg of 5-FU and plasma samples were obtained 23–48 hours after administration to measure the β-phase (table 2). The plasma elimination half-life during this period was found to be about 20 hours in both rats. Parallel decay curves for total concentration of 5-FU metabolites measured by plasma C^{14}-radioactivity and for 5-FU measured by mass fragmentography indicated equilibrium between 5-FU and its anabolites. Degradative metabolite levels should also parallel 5-FU levels, assuming that their rate of elimination is faster than their rate of formation (ie, half-life < 20 hours).

Plasma Levels of 5-FU in Man After Iv and Oral Administration

Figure 4 demonstrates plasma levels of 5-FU as measured by mass-fragmentography in three patients after iv administration at 15 mg/kg of 5-FU. Plasma concentrations of 5-FU were similar in these three patients over about 13 half-lives and started to level off in two patients 2 hours after administration. The initial half-life of elimination was 12 minutes with an extrapolated zero-time concentration of about 40 μg/ml of 5-FU representing a volume of distribution of about 38% body weight. The integrated areas under the plasma concentration-time curve were 13, 15, and 21 μg × hr/ml, respectively.

TABLE 2.—Plasma concentrations 23–48 hours after 90 mg/kg of 5-FU was injected into two rats

Hours after 5-FU dose	Total C^{14} (μg/ml)	Total 5-FU (μg/ml)
23	1.0, 1.5	0.05, 0.045
30	0.8, 0.7	0.035, 0.04
48	0.4, 0.4	0.015, 0.02

5-FU plasma concentrations after oral administration of 15 mg/kg of 5-FU are depicted in figure 5. Peak plasma levels of 5-FU were obtained between 10 minutes and 2 hours after administration and ranged between 0.8 and 60 μg/ml. The patient with the highest peak level (60 μg/ml after 10 minutes) showed an initial log-linear elimination phase with a half-life of about 10 minutes. At 0.6 μg/ml of 5-FU, plasma concentrations started to level off into the β-phase. Plasma levels were below the detectable

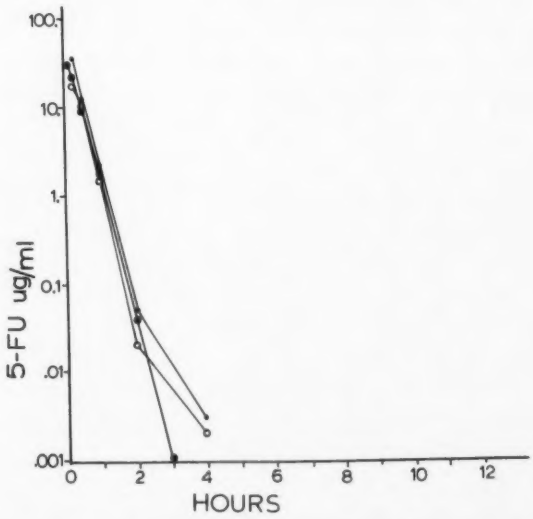

FIGURE 4.—5-FU plasma concentrations in man after iv doses of 15 mg/kg of 5-FU. •——• = Patient No. 1, ○——○ = patient No. 2, ●——● = patient No. 3.

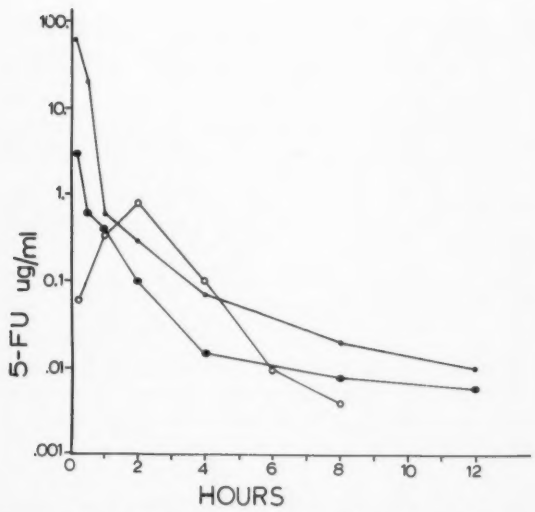

FIGURE 5.—5-FU plasma concentrations in man after oral doses of 15 mg/kg of 5-FU. •——• = Patient No. 4, ○——○ = patient No. 5, ●——● = patient No. 6.

limit 24 hours after administration. Plasma levels of the other two patients also persisted at measurable levels for over 8–12 hours after administration. The integrated areas under the plasma concentration-time curves were 26 μg × hr/ml (peak level, 60 μg/ml), 1.5 μg × hr/ml (peak level, 3 μg/ml), and 1.7 μg × hr/ml (peak level, 0.8 μg/ml).

DISCUSSION AND CONCLUSIONS

This study represents an attempt to utilize a specific and highly sensitive analytical assay of 5-FU plasma levels in the pharmacokinetic evaluation of 5-FU treatment of cancer patients. Using this technique we have found a β-phase of 5-FU elimination from plasma after high iv doses in rats and therapeutic oral doses in patients with rapid gastrointestinal absorption, which supports the proposed two-compartmental open model of 5-FU plasma disposition. Iv administration of 90 mg/kg of 5-FU in rats resulted in a measurable β-phase with a half-life of about 20 hours. This long half-life can almost certainly be related to a rapid metabolic equilibrium between free 5-FU which distributes rapidly throughout the body and its anabolic pool with mostly intracellular location. Assuming that all metabolic and diffusion processes are first order resulting in linear kinetics, a reduction of the dose by one half should lower 5-FU plasma concentrations accordingly. Since measured 5-FU plasma levels at 40 and 15 mg/kg in rats were found to be lowered by a factor of 10 or more in the β-phase, nonlinear kinetics can be assumed. Variation of the dose level did not significantly affect the volume of distribution. Presumably, saturation of the degradative metabolism occurred at the high dose level as reported elsewhere (4), which would divert a larger fraction of the 5-FU dose into the anabolic pool. This may be correlated to the clinical observation that doses of 5-FU above 20–30 mg/kg demonstrate an unproportional increase in toxicity (14,15). After oral 5-FU doses, large variations in peak plasma levels were found in three patients which represent extremes of published 5-FU levels after oral administration (2,9,12). Considering the very rapid elimination of 5-FU, only small changes in the rate of absorption result in large changes of peak 5-FU plasma concentrations. Two patients with very rapid absorption showed a measurable β-phase of plasma elimination, which may be an indication of a larger anabolite pool size in these patients when compared to anabolite pools in patients receiving iv doses. These data suggest a hepatic first-pass effect on systemic bioavailability of 5-FU as well as formation of active anabolic metabolites, which may depend upon the rate of absorption. Further studies could provide a pharmacokinetic rationale for monitoring patients under 5-FU treatment.

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"Platinum-Pyrimidine Blues" and Related Complexes: A New Class of Potent Antitumor Agents[1,2]

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SUMMARY

The diaquo species of *cis*-dichlorodiammineplatinum (II) reacts with pyrimidines and substituted pyrimidines to form very soluble, deep blue complexes. These complexes are believed to consist of mainly monomeric species containing one pyrimidine moiety liganded to each platinum. The structures of the complexes are largely uncertain at this time. We describe the methods of synthesis and characterization of approximately 70 such complexes, with a suggested classification scheme, including other types of "platinum blue" complexes formed with acetamides. Many of these complexes show superior activity against the ascites Sarcoma 180 tumor in Swiss mice when compared to *cis*-dichlorodiammineplatinum (II). Initial screening test results and dose schedule-dependency results indicate we can achieve 100% cures in this tumor-host system. Activity is also shown against the Rauscher leukemia, Ehrlich ascites, and ADJ/PC6A tumors. Microscopic histopathologic studies of resulting kidney lesions show that the "platinum-uracil blue" complex causes only minor focal damage to the proximal convoluted tubules at a therapeutic dose level, rather than the generalized degenerative changes so prominent and dose limiting noted with *cis*-dichlorodiammineplatinum (II).

[Cancer Chemother Rep 59:287–300, 1975]

In the course of an investigation of the mechanisms of the antitumor activity of *cis*-dichlorodiammineplatinum (II)[7] we have studied the interactions of this drug and its aquation products with nucleotides and polynucleotides. One of us (S. Mansy) noted that the aquated products underwent a slow reaction with polyuracil yielding a deep blue solution.[8]

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[5] It is a pleasure to express our gratitude to Mr. G. A. Moldovan and Mr. V. A. Polly for their assistance in the chemical syntheses, to Mrs. B. Rabin for assistance in the animal tests, to Drs. T. A. Connors, G. Gale, H. Ridgway, and R. Speer for their generosity in allowing us to quote their unpublished results in other animal tumor systems, and to Dr. J. Stanko for running the ESCA measurements for us.

[6] *Reprint requests to:* Dr. Barnett Rosenberg, Department of Biophysics, Michigan State University, East Lansing, Mich 48824.

[7] *Cis*-dichlorodiammineplatinum (II): NSC-119875; CAS reg. No. 15663-27-1.

[8] Mansy S, Rosenberg B, and Getty R. The interaction of diaquodiammineplatinum (II) isomers with polynucleotides in solution determined by Laser-Raman scattering. Manuscript submitted for publication, 1973.

Evaporation to dryness produced a dark blue, amorphous powder with a very high solubility in water. Subsequently, we showed that uracil can replace polyuracil, with the same result. Because of the very high solubility of this product (~ 50 g/liter) and because it contained the core of an active antitumor drug, we decided to test it for antitumor activity. Surprisingly, the results were quite good: the first tests showed a marked superiority of cure rates of the ascites Sarcoma 180 in Swiss mice compared to *cis*-dichlorodiammineplatinum (II). Later, autopsy results suggested that the "platinum-uracil blue" adduct did not produce the generalized kidney toxicity so dominant in all other active platinum complexes.

These three preliminary results (higher activity than *cis*-dichlorodiammineplatinum (II), very high solubility, and lack of dose-limiting kidney toxicity) recommended this adduct as a valuable improvement over other platinum complexes. Therefore, we undertook a survey of this new group of complexes to determine the breadth of congeners we could synthesize and to evaluate the antitumor activities of these against our standard test tumor, the ascites Sarcoma 180. We report here the first results of this survey.

CHEMISTRY OF THE "PLATINUM BLUES"

While blue compounds of platinum with acetamide were discovered in the late 19th (1) and early 20th centuries, the chemistry of these and the new class of "platinum-pyrimidine blues" is little understood. To add clarity to our discussion of these new antitumor drugs, we have separated them into a number of different classes, given below, which vary according to starting materials and reaction schemes. First, however, we will present a short historical introduction to these anomalously colored platinum complexes.

Credit for the discovery of "Platinblau," as these blue complexes were designated, is usually given to Hofmann and Bugge (2). They reacted Ag_2SO_4 with the yellow platinum (II) species, $Pt(CH_3CN)_2Cl_2$, in aqueous solution and isolated a deep blue, amorphous material. This material was thought to be monomeric in nature, containing platinum in the divalent state. Since this discovery, only a few papers have appeared concerning further studies on "Platinblau" and similar blue products. Gillard and Wilkinson (3) postulated that "Platinblau" had the empirical formula $Pt(CH_3CONH)_2 \cdot H_2O$ with polymeric chains, bridging acetamide groups, and divalent platinum. Others (4,5) have attempted to demonstrate that it is a platinum (IV) complex containing chelating acetamide ligands and hydroxyl groups in the other two coordination positions. In our laboratory, experiments were performed which established that both blue and purple products could be isolated from the "Platinblau" reaction, with the purple species being the more highly oxidized (vide infra). Thus, there is considerable controversy over the exact nature of this complex. The important point we stress is that regardless of its exact chemical nature, "Platinblau" and similar compounds prepared using different alkyl nitrile complexes of platinum as starting materials are potent antitumor agents.

Researchers (4,5) have reported the preparation of highly colored amide complexes of platinum by heating, for example, trimethylacetamide and either $Pt(CH_3CN)_2Cl_2$ or $K_2[PtCl_4]$ (a reddish salt). From this reaction three components were identified by chromatography. These were two yellow crystalline materials and a blue amorphous powder. Although they reported that they could not identify any of these products in a positive manner, they postulated that the blue material contained tetravalent platinum, with bidentate amide anions and chloride ligands completing the coordination sphere. We have prepared similar complexes with various substituted acetamide ligands. The results of chemical analyses and tests of these complexes for antitumor activity are given in this report.

We report here entirely new and different types of "platinum blue" complexes which are prepared from reactions of neutralized *cis*-diaquodiammineplatinum (II) and uracil, its substituted derivatives, and various amides. These are noncrystalline materials for which we have been able to obtain little structural information at this time. The only other reference to anomalously colored platinum compounds containing *cis*-amino groups as

ligands rather than amides is that concerning mixtures of *cis*-dichlorodiammineplatinum (II) and sulfuric acid (3). Crystals of this blue-black material were obtained and a preliminary X-ray diffraction study showed that the platinum-platinum distance was 3.06 A, suggesting strong interaction. It was concluded that this complex contained layers of *cis*-dichlorodiammineplatinum (II) held together by platinum-platinum bonds, with the sulfate ion hydrogen bonded to the coordinated ammonia groups. However, in our "platinum-uracil blues" and related compounds there are no chloride ions bonded to platinum. We can only assume that the 2,4-dihydroxypyrimidine bases are coordinated, with the blue color arising from perturbations of the platinum electronic energy levels due to ligand field interactions.

We will present here a detailed description of the preparation and characterization of the many different types of green, blue, or purple platinum complexes which are effective antineoplastic agents. The chemistry of these drugs is complicated due to the uncertain structures, which we are now attempting to elucidate.

CLASSIFICATION SCHEME OF THE "PLATINUM BLUES"

In the absence of exact knowledge of the structures of the "platinum blue" complexes prepared and tested here, we will arbitrarily divide them into three classes on the basis of their general mode of preparation. While the complexes contained within each class seem to have somewhat analogous structures and similar chemical properties, we emphasize that the various classes refer *only* to the mode of preparation. It is possible that complexes in the same class may actually have vastly different structures. The classes are described below.

Class I.—These complexes are prepared by the reaction shown in equation 1:

$$[cis\text{-}Pt(NH_3)_2(H_2O)_2]^{+2} + \text{"amide"} \xrightarrow[\substack{H_2O \\ 37^\circ \\ 2\text{-}21\ days}]{pH7} \text{Class I complex} \qquad (1)$$

The "amide" in this equation may be a primary alkyl or aryl amide, a hydantoin, or any of a large number of 2,4-dihydroxypyrimidines. The class I complexes are further divided into class IA complexes, which are blue and less soluble in water-ethanol mixtures, and class IB complexes, which are green and more soluble. The *cis*-diammino configuration of platinum appears to be essential for the formation of these complexes. Attempts to prepare class I complexes from the corresponding *trans*-isomer or from *cis*-species with substituted or chelating amines have not yet been successful; however, tan species have been isolated and their antitumor activity will be discussed in a later publication.

Class II.—These are the classic "Platinblau" complexes formed by the reactions shown in equations 2A or 2B.

$$Pt(R-CN)_2Cl_2 + 2AgNO_3 \xrightarrow[2\text{-}5days]{H_2O} \text{Class II complex} \qquad (2A)$$

$$\text{Class III complex} + 2AgNO_3 \xrightarrow[2\ days]{H_2O} \text{Class II complex} \qquad (2B)$$

Of the complexes we have synthesized, those prepared from the acetonitrile and propionitrile platinum complexes are water soluble and are effective in our tumor system. The reaction apparently proceeds to give some form of amido complex (vide infra).

Class III.—These are the halogen-containing "Platinblau" complexes originally prepared by Brown et al (4,5). Three different modes of preparation were used, as shown in equations 3A, 3B, and 3C. The reaction in equation 3A is the only one previously reported.

$$Pt(CH_3CN)_2Cl_2 + R-\overset{O}{\overset{\|}{C}}-NH_2 \xrightarrow{fuse} \text{Class III complex} \qquad (3A)$$

$$Pt(CH_3CN)_2Cl_2 + R-\overset{O}{\overset{\|}{C}}-NH_2 \xrightarrow[H_2O]{\Delta} \text{Class III complex} \qquad (3B)$$

$$Pt(R-CN)_2Cl_2 \xrightarrow[H_2O]{\Delta} \text{Class III complex} \qquad (3C)$$

These three reactions appear to give the same products, but the chemistry of these reactions is apparently quite complex.

PREPARATION OF THE COMPLEXES

Class I

All of the class I complexes containing 2,4-

dihydroxypyrimidines were prepared and isolated by the same general method; the preparation of the class I uracil complexes (A and B) will be used as examples. In a typical preparation, 3.0 g (0.01 mol) of *cis*-dichlorodiammineplatinum (II) (supplied by Matthey Bishop, Inc., or Engelhard Industries) and 3.4 g (0.02 mol) of silver nitrate were stirred overnight in about 100 ml of water to produce about a 0.1 M solution of *cis*-diaquodiammineplatinum (II) nitrate. The silver chloride precipitate was filtered off and a small aliquot of the supernatant liquid was tested for silver ion with 0.1 N HCl. The absence of silver ions appears to be necessary for the reaction to proceed. The platinum-containing solution was brought to pH 6.5–8.0 by addition of 2 M NaOH. Uracil (1.12 g, 0.01 mol) was added to 100 ml of water and dissolved by heating and addition of sufficient 2 M NaOH to bring the pH to about 9. The uracil and platinum solutions were mixed and the pH was readjusted to 7–8. The solution flask was then placed in a 37°–40°C water bath and covered with aluminum foil for 3 days to 2 weeks. The solution usually becomes blue in the uracil case within 24 hours. Some of the other complexes are considerably slower, the blue color appearing in 3–8 days. After the solution had become dark blue, and some blue precipitate had formed, the solution was cooled to 0°C overnight and the blue solid collected by filtration. The solid was washed with a little cold water and then with copious quantities of boiling ethanol. Reprecipitation of the dark blue powder from water by the addition of ethanol gave the class IA uracil complex as a dark blue powder. The supernatant liquid from the first filtration was evaporated to about 25% of its original volume and an equal volume of ethanol was added. Cooling to 0°C gave more blue precipitate and a dark green solution. After filtration and further concentration, addition of more ethanol gave a pale green precipitate. This was washed with boiling ethanol to give the class IB uracil complex as a pale green powder. The class I complexes containing hydantoin or primary amides were prepared by an analogous procedure except that it was not necessary to add NaOH to the amide solutions to attain a neutral pH.

In addition, we have been informed that the reaction of *cis*-dichlorobiscyclopropylamineplatinum (II) with 1-methylthymine forms a purple cationic complex which has shown some antitumor activity against the ADJ/PC6A system.[9] The workers reporting this development were unable to repeat the reaction with different *cis*-dichlorodiammineplatinum (II) complexes or other substituted pyrimidines. We have verified formation of the purple compound in solution but have not tested it against our animal system as yet.

Class II

These complexes were prepared by the method of Gillard and Wilkinson (3) from the appropriate dichloro(bisorganonitrile)platinum (II) complexes and silver nitrate. After the blue-black solutions had formed, the mixtures were allowed to react at 37°C for 4–6 days. These solutions were then evaporated to dryness and the solids collected and purified. With $Pt(CH_3CN)_2Cl_2$ as the starting material the solid from the above reaction contained a purple acetone-soluble fraction (class IIB acetamide complex) and a blue acetone-insoluble part (class IIA acetamide complex). The class IIA chloroacetamide and fluoroacetamide complexes were prepared from the respective class III complexes (*see* below) by reaction of one equivalent of the class III complex with two equivalents of silver nitrate in a 30% ethanol-water mixture. These products were insoluble in the solvent mixture so the precipitate was isolated by filtration and then extracted with acetone to give a blue solution. Addition of water gave a black precipitate. The class IIA fluoroacetamide and chloroacetamide complexes were obtained as black powders by filtration.

Class III

We could repeat the preparation of "platinum blue" complexes by fusion of dichlorobisacetonitrileplatinum (II) with an organic amide as reported by Brown et al (4,5) only when the amide was acetamide. Attempts to prepare other complexes by this method resulted in apparent

[9] Thomson AJ, and Roos IAG. Personal communication.

decomposition without any blue product formation. However, we found that the reaction proceeded smoothly if carried out in aqueous solution. This is most useful in the preparation of water-insoluble complexes since hydrolysis products of the starting platinum complex, $Pt(CH_3CN)_2Cl_2$, are water soluble. These class III acetamide complexes were prepared both by fusion and by heating dichlorobisacetonitrile-platinum (II) in the presence and absence of acetamide. The product obtained in the absence of added acetamide seems to be the only one with high activity against ascites Sarcoma 180 in mice.

When the blue solutions from aqueous preparations were evaporated to dryness in a vacuum, a red solid was formed. This solid material was dissolved in acetonitrile, and ether was added giving a red oil. Scrubbing of the oil with ether gave the red complex as a dark red-black hygroscopic powder.

ANALYSES OF THE "PLATINUM BLUES"

Chemical analyses were performed by Galbraith Laboratories (Knoxville, Tenn) and Spang Microanalytical Laboratory (Ann Arbor, Mich). The analytic data are compiled in table 1. The results of the analyses are somewhat variable, presumably due to the fact that many of these products are mixtures (by thin-layer chromatography) that are very difficult to separate. However, a number of conclusions can be drawn about these complexes. The class I complexes containing 2,4-dihydroxypyrimidines contain one pyrimidine per platinum in all cases. Most of the analyses fit rather closely the formulation of these complexes as containing two ammonia ligands, one pyrimidine anion, and one hydroxide ion per platinum but with two additional oxygen atoms at an unspecifiable location. The 5-fluorouracil complex is an exception in that it fits the above formulation exactly without excess oxygen. The two extra oxygen atoms could be accommodated in the analyses if the complexes were formulated as containing platinum (IV) with three hydroxyl ligands; however, this would conflict with some of the physical data (see Appendix). Table 1 compares calculated analytic results for a structure of this type with those found experimentally. Since total analyses of all elements except platinum were determined, the percentage platinum in each complex is listed by difference.

TABLE 1.—Elemental analyses of representative class IA complexes

Pyrimidine	Postulated formula		Percent of—						
			C	H	N	O	Cl	F	Pt
Uracil	$PtC_4H_{12}N_4O_8$	Calculated	12.28	3.09	14.32	20.45	—	—	49.86
		Found	12.51	2.52	14.52	20.37	—	—	50.02
Thymine	$PtC_5H_{14}N_4O_8$	Calculated	14.81	3.48	13.82	19.73	—	—	48.12
		Found	14.05	3.26	15.65	18.97	—	—	48.07*
1-Methyluracil	$PtC_5H_{14}N_4O_8$	Calculated	14.81	3.48	13.82	19.73	—	—	48.12
		Found	14.65	2.63	14.08	19.77	—	—	48.87*
5,6-Dihydro-6-methyluracil	$PtC_5H_{16}N_4O_8$	Calculated	14.74	3.94	13.76	19.64	—	—	47.90
		Found	13.07	3.36	15.60	17.21	—	—	50.76*
5,6-Dimethyluracil	$PtC_6H_{16}N_4O_8$	Calculated	17.18	3.85	13.36	19.08	—	—	46.53
		Found	16.96	3.38	15.07	15.78	—	—	48.81*
5-Chlorouracil	$PtC_4H_{11}N_4O_8Cl$	Calculated	11.28	2.60	13.16	18.79	8.33	—	45.83
		Found	10.16	2.17	14.91	17.98	8.76	—	46.02*
6-Chlorouracil	$PtC_4H_{11}N_4O_8Cl$	Calculated	11.28	2.60	13.16	18.79	8.33	—	45.83
		Found	11.24	2.45	13.37	18.63	8.28	—	46.03*
5-Fluorouracil	$PtC_4H_9N_4O_8F$	Calculated	12.80	2.42	14.93	—	—	5.06	—
		Found	12.65	2.26	14.75	—	—	5.09	—

*Determined by difference.

ANTITUMOR ACTIVITY OF THE "PLATINUM BLUES"

The chemotherapeutic activities of the "platinum blue" complexes were determined using the ascites Sarcoma 180 tumor in Swiss mice. The protocols for these tests were as follows. Randombred female Swiss mice (Spartan Laboratory, Williamston, Mich), 18–20 g in weight, were randomized in groups of six. The ascites cells were removed from the peritoneal cavities of animals with approximately 10-day-old ascites tumors. The cells were washed several times with 0.85% saline and spun down each time in a refrigerated centrifuge for 3–5 minutes at 750 rpm. After the blood was removed from the cells, they were diluted with 0.85% saline solution and counted in a hemocytometer. A final dilution of 2×10^7 cells/ml in saline was made. Each animal received 0.2 ml of this suspension (4×10^6 cells/animal). This was injected intraperitoneally (ip) on Day 0 of each test. Two groups (12 animals) were kept as untreated controls, and the test was terminated at twice the mean day of death of the untreated controls. Two groups (12 animals) were the positive controls which were injected ip on Day 1 with a solution of *cis*-dichlorodiammineplatinum (II) in saline at a dose of 7 mg/kg. All test compounds were injected ip on Day 1 as a 0.5-ml volume of the compound in a carrier of water, physiologic saline, or arachis oil (peanut oil). Soluble compounds were always tested in appropriate solutions; insoluble compounds were tested as a slurry. The slurry was sonicated for periods of up to 10 minutes to insure uniformity of dispersion just prior to injections. Generally, four dose levels, in a doubling escalation, were tested for each compound in the original survey. Retests were performed if we had not reached a sufficient level of drug toxicity at the highest dose. Animals still alive at the completion of the tests were considered to have died on the day of evaluation. Animals showing no abdominal distention on this day (by palpation or visibly) were considered cured. Some cured animals later developed a solid tumor around the site of injection of the cells. We believe this was due to a leakage of cells along the route of the needle on injection or a later leak of cells through the injection hole in the peritoneum. In any case, we consider this an adventitious effect, which, when care is used in injections and smaller needles are used, becomes negligible.

The compounds tested were usually freshly prepared and purified. Some compounds were kept for further testing, and we now store these in a vacuum dessicator in a dark refrigerator to prevent deterioration. In general, compounds of all classes tested had toxic levels at 200 mg/kg or greater, with the exception of the alkyl acetamide complexes of classes IA and IB. A toxic level is considered to be that concentration where two or more of the six animals at the dose died within 8 days of injection. Animals dying after this time overlap with the early deaths of the untreated controls, and since we did not perform gross autopsies on a routine basis, we could not clearly specify the causes of death.

For approximately one third of the complexes tested, the animals exhibited an excessive extension of the hind legs shortly after injection at high dose levels. The animals surviving the first 2 days, however, usually survived past the 8-day limit for toxic deaths. Some, however, did die early. Consistent with our early experience with platinum complexes, gross hepatic damage was minimal or non-existent (with the exceptions of $Pt(CH_3CN)_2Cl_2$, the platinum-1-methyluracil [class I], the platinum-5-bromo-1-methyluracil [class I], and the platinum propionamide [class IIA]). Peritonitis occurred in a similar number of cases at later times (day of evaluation, and up to 3 months later in cured animals). Symptoms of neuromuscular disorders were observed with a few compounds shortly after high-dose injections (ie, platinum-uracil, class IB). No symptoms of central nervous system disorders were ever observed in the test animals.

Unlike the solid Sarcoma 180, the ascites tumor in the Swiss mice elicits no spontaneous regression (no survivors among 336 animals), all mice with tumors die, and the percent of no-takes is zero. The mean day of death is 17.5, with a small standard deviation (± 2.2 days).

The results of the survey tests are presented in table 2. We grouped the compounds according to class as described above. The data shown

TABLE 2.—Survey results of antitumor activity of "platinum blue" complexes against ascites Sarcoma 180 in Swiss mice*

Compound	Carrier†	Dose range (mg/kg)	Toxic level (mg/kg)	Best %ILS	Dose of best %ILS (mg/kg)	Physical state‡	No. of cures§
Positive control [*cis*-dichlorodiammineplatinum (II)]	S	7	10	49 (SD ± 2.82)	7	S	1 (of 12)
Class IA¶							
Uracil	W	50–400	400	91	200	S	5
Uracil	S	50–400	400	80	100	S	1
5,6-Dihydrouracil	W	20–800	400	92	200	S	4
Thymine	W	150–600	450	72	300	S	2
Thymine	S	50–200	>200	67	150	S	1
5,6-Dihydro-6-methyluracil	W	50–400	200	89	50	S	2
6-Methyluracil	S	200–800	>800	87	600	S	3
5,6-Dimethyluracil	W	50–400	>400	100	400	S	5
5,6-Dihydrothymine	S	50–400	400	87	200	S	2
1-Methyluracil	S	50–400	>400	85	400	S	3
1-Methylthymine	S	50–400	400	94	100	S	3
1–Ethyluracil	S	50–400	200	38	50	S	0
5-Fluorouracil	W	50–400	200	90	100	S	4
5-Chlorouracil	W	50–400	200	67	50	S	3
6-Chlorouracil	S	25–200	>200	88	200	S	3
5-Bromo-1-methyluracil	S	50–400	>400	88	200	S	1
5-Iodouracil	PO	25–600	500	8	250	Sl	0
5-Hydroxymethyluracil	S	50–400	200	98	100	S	2
5-Carbethoxyuracil	S	25–200	200	56	200	S	0
6-Carbomethoxyuracil	S	25–200	>200	38	25	S	0
Uridine deoxyribose	S	25–200	>200	46	200	S	0
Thymidine	S	50–400	200	46	50	S	1
5-Iodouridine deoxyribose	PO	50–200	>200	23	200	Sl	0
2′,3′,5′-Triacetyluridine	S	50–1000	800	79	600	S	2
2′,3′,5′-Tribenzoyluridine	S	50–400	>400	19	100 and 200	S	0
2′,3′-Isopropylidineuridine	S	50–400	400	61	50	S	2
Acetamide	S	25–200	25	−94	25	S	0
Trimethylacetamide	S	3.1–25	12.5	10	6	S	0
3-Chloropropionamide	S	3.1–25	>25	61	25	S	2
Benzamide	S	50–400	400	23	400	Sl	0
Class IB							
Uracil	W	2[illegible] 375	500	95	340	S	4
Thymine	S	50–400	>400	60	200	S	0
1-Methylthymine (yellow)	S	50–400	400	−14	100	S	0
1-Ethyluracil	S	50–400	100	15	50	Sl	0
5-Fluorouracil	S	25–200	>200	37	200	S	0
Hydantoin	S	25–200	>200	60	50	S1	1
Benzamide	S	3.1–25	25	14	3.1 and 6.2	S	0
Trimethylacetamide	S	3.1–25	3.1	−55	3.1	S	0

TABLE 2.—Survey results of antitumor activity of "platinum blue" complexes against ascites Sarcoma 180 in Swiss mice*—Continued

Compound	Carrier†	Dose range (mg/kg)	Toxic level (mg/kg)	Best %ILS	Dose of best %ILS (mg/kg)	Physical state‡	No. of cures§
Class IIA							
Acetamide	W	100–400	>400	93	200	S	1
Fluoroacetamide	S	50–400	>400	94	400	S	0
Chloroacetamide	S	50–400	>400	96	400	S	1
Propionamide	2% EtOH	50–400	>400	89	200	S	2
n-Butyramide	S	50–400	>400	23	100	Sl	0
Benzamide	S	50–400	>400	14	100	Sl	0
Class IIB							
Acetamide	S	50–400	400	91	200	S	3
Class III							
Acetamide (purple)	S	50–400	400	31	400	S	1
Acetamide (blue)**	S	50–200	200	76	150	Sl	0
Acetamide (blue)	S	25–200	200	39	100	S	0
Fluoroacetamide	S	50–400	400	66	400	Sl	0
Chloroacetamide	S	50–400	400	53	200	Sl	0
Trimethylacetamide	S	50–400	400	67	50	S	0
Acetamide (red)	S	6.3–400	100	91	25	S	2
Miscellaneous							
$Pt(CH_3CN)_2Cl_2$	S	6.3–50	50	83	25	S	1

*Average day of death of untreated controls = 17.5 (SD ± 2.16).

†W = water, S = saline, PO = peanut oil.

‡S = solution, Sl = slurry.

§Six animals per test; cures are considered as having *no* distention of abdominal cavity but do include formation of solid tumors at site of injection in some cases. Single injections given ip on Day 1 only.

¶Refer to section on Preparation of the Complexes for a description of this class.

**Prepared by hydrolysis of $Pt(CH_3CN)_2Cl_2$.

include the carrier, the dose range tested, the toxic level (*see* above), the best percent increase in lifespan (%ILS; the maximum being 100% since the experiments were terminated at twice the mean lifespan of the untreated controls), the dose level giving the best %ILS, the physical state of the inoculum (solution or slurry), and the number of cured animals (out of six in each group).

It can be seen that each class contains members with high activity (%ILS > 50) and others with low activity. As a general rule, therefore, antitumor activity is not strongly correlated with our somewhat arbitrary "class" designations. Substitutions can be made on the pyrimidines in the 1, 5, and 6 positions and the resulting blue complexes still show high activities. Large groups, such as the sugars and substituted sugars (ie, thymidine, uridine, 2′,-3′,5′-triacetyluridine, etc), can be added to the N-1 position without much change in the characteristics (chemical and biologic) of the complexes.

A rough correlation can be made between low toxic levels (high toxicities) and low %ILS values (ie, acetamide [class IA], benzamide [class IB], trimethylacetamide [class IB], etc). The converse is not true, that is, high toxic levels (low toxicities) do not guarantee high antitumor activities.

Since substituted pyrimidines form a group of potent antitumor agents by themselves (antimetabolites), it is possible that at least some of the above results are due to this. We can eliminate this as a possibility in at least one case, that of 5-fluorouracil,[10] which by itself is inactive in our tumor-host system, while the platinum-5-fluorouracil blue complex is very

[10] 5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

active and much less toxic. The data supporting this are shown in table 3.

The results in table 2 were obtained using a single ip injection on Day 1. Since this may not be the best schedule for treatment we have selected samples of class I and II drugs for schedule-dependency tests. These are described in table 4. *Cis*-dichlorodiammineplatinum (II) given in eight injections of 1 mg/kg each every 3 hours for the first day showed a surprisingly improved result over the single injection of 7 mg/kg (positive control). Earlier tests on a few other tumor systems did not indicate such a strong dose schedule dependency. Also, as discussed below, the kidney toxicity was greatly minimized using the divided dose. These results suggest that clinical trials of this drug with a 24-hour divided dose schedule (a schedule not encompassed by any of the present phase I or II clinical trials) should be attempted. In the case of the "platinum blues," a slight superiority in results is obtained using the various divided dose schedules over a single injection. This is also evident in table 5. In further testing of these "platinum blues," we recommend the inclusion of a divided dose schedule (every day for 5 days or every fifth day).

TESTS OF ACTIVITY AGAINST OTHER TUMOR SYSTEMS

To determine the spectrum of activity of the "platinum blues" against tumor systems other than ascites Sarcoma 180, we have, at times, submitted samples of our preparations to other laboratories for such testing. Tables 5–8 present the results of the "platinum blues" tested against L1210 leukemia, MCDV-12 leukemia (Rauscher leukemia, virus-induced), Ehrlich ascites carcinoma, and ADJ/PC6A (myeloma) tumor.

It is obvious that the results of tests in the L1210 tumor system (table 6) indicate little or no activity. This compares poorly with a 100% ILS and occasional cures obtained by *cis*-dichlorodiammineplatinum (II) at maximally therapeutic doses averaged over a large number of tests. The L1210 system, we conclude, is only marginally responsive to any of the "platinum blues" tested so far. We recommend that this would be a poor system for screening further compounds of this nature. The quite good results obtained in the Rauscher leukemia tests (table 5), however, do not allow any generalizations concerning lack of activity against leukemias. It would be instructive to compare the activities of other chemotherapeutic agents against these two tumor systems, to determine if any other classes of agents show such discordant results. This may provide a clue to the mechanism of action of the "platinum blues."

With regard to the Ehrlich ascites and ADJ/PC6A tumors, no clear superiority of the "platinum blues" over *cis*-dichlorodiammineplatinum (II) is evident but high activity is shown for both. The LD 50s shown in table 7 are high, consistent with our results and similar to those

TABLE 3.—Comparison of the antitumor activity of a substituted pyrimidine (5-fluorouracil) and its "platinum blue" complex against ascites Sarcoma 180

Compound	Carrier	Dose (mg/kg)	No. of toxic deaths	%ILS	Physical state	No. of cures†
5-Fluorouracil	Water	20	0	0	Solution	0
	Water	40	0	4	Solution	0
	Water	60	0	8	Solution	0
	Water	80	0	–18	Solution	0
$Pt(NH_3)_2$ (5-fluorouracil), class IA*	Water	50	0	49	Solution	0
	Water	100	0	90	Solution	4
	Water	200	6	–79	Solution	0
	Water	400	6	–94	Solution	0

*Refer to section on Preparation of the Complexes for a description of this class.

†Six animals per test; cures are considered as having *no* distention of abdominal cavity but do include formation of solid tumors at site of injection in some cases. Single injections given ip on Day 1 only.

TABLE 4.—Results of schedule-dependency tests of antitumor activity of selected "platinum blue" complexes against ascites Sarcoma 180*

Compound†	Dose schedule	No. of injections	Carrier	Dose range (mg/kg/injection)	Toxic level (mg/kg)	Best %ILS	Dose of best %ILS (mg/kg/injection)	Physical state‡	No. of cures§
Positive control [*cis*-dichlorodiammine-platinum (II)]	Day 1	1	Saline	7	10	42 (SD ± 3.1)	7	S	1
Cis-dichlorodiammine-platinum (II)	q3h	8	Saline	1	>1	86	1	S	5
	Daily	5	Saline	3	3	19¶	3	S	3
	q5d	5	Saline	5	>5	88	5	S	3
Uracil, class IA	q3h	8	Saline	50–125	100	80	50	S	1
	Daily	5	Saline	50–150	>150	100	100	S	6
	q5d	5	Saline	75–300	>300	100	150	S	3
Uracil, class IB	q3h	4	Saline	100–250	>250**	73	100	S	3
	Daily	5	Saline	25–200	200	68	100	S	5
	q5d	5	Saline	100–400	400	84	200	S	5
Thymine, class IA	q3h	8	Saline	50–200	200**	77	100	S	4
	Daily	5	Saline	50–150	150**	91	50	S	5
	q5d	5	Saline	75–300	300	100	150	S	5
Acetamide, class IIA	q3h	8	Saline	50–200	100	−12	50	Sl	0
	Daily	5	Saline	50–200	>200	37	200	Sl	0
	q5d	5	Saline	50–200	>200	61	150	Sl	0
Propionamide, class IIA	q3h	8	Saline	50–200	>200	28	200	Sl	1
	Daily	5	Saline	75–300	225	28	150	Sl	1
	q5d	5	Saline	50–400	>400	51	50	Sl	1

*Average day of death of untreated controls = 19.3 (SD ± 3.2).

†Refer to section on Preparation of the Complexes for a description of the classes.

‡S = solution, Sl = slurry.

§Six animals per test; cures are considered as having no distention of abdominal cavity but do include formation of solid tumors at site of injection in some cases.

¶Three animals died within 9 days of start of treatment; remainder were cured.

**No. of injections varied because of severe animal response. Therefore, toxicity probably would have been reached had all animals received the same No. of injections.

for the cycloalkylamine platinum complexes reported by Connors et al (6), but the ID90 values are much higher, thus yielding the lower therapeutic indicies.

We tentatively conclude from these early results that the "platinum blues" may have activity against a broad spectrum of tumors, as does *cis*-dichlorodiammineplatinum (II).

One caveat is necessary here, and that is that we have now found that some of the "platinum blues" are unstable with time under ordinary storage conditions. This leads to a decreased solubility, a higher toxicity, and a lower activity against the ascites Sarcoma 180 tumor. Some deterioration may have occurred between syntheses in our laboratory and testing in these other laboratories. This is suggested by the somewhat higher toxicities shown in tables 5–8 compared to our results shown in table 2. We have found that storage in a refrigerated desiccator in the dark has produced no deterioration over a 3-month period. Until the cause of the instability is determined, we suggest that all such compounds be stored in this manner.

COMPARATIVE MICROSCOPIC RENAL DAMAGE OF VARIOUS PLATINUM COMPLEXES

Renal toxicity is the dose-limiting side effect in higher animals and man of treatment with *cis*-dichlorodiammineplatinum (II). It is desirable to find other platinum drugs which cause much less severe renal toxicity. We describe here the results of histopathologic examinations indicating that "platinum-uracil blue" (class IA) causes far less impairment of the kidneys than does *cis*-dichlorodiammineplatinum (II) at comparable therapeutic levels.

The protocols for these tests were as follows. Each group contained six female Swiss mice; the tumored animals were given a transplant of solid Sarcoma 180 on Day 0 and treatment was initiated on Day 1. The animals were killed

TABLE 5.—Effects of *cis*-dichlorodiammineplatinum (II) and "platinum blues" on MCDV-12 leukemia (Rauscher leukemia, virus-induced) in BALB/c mice*

Compound	Dose (mg/kg)	Schedule	%ILS	No. of survivors/ No. treated
Cis-dichlorodiammine-platinum (II)	5	Once	146	2/3
	8	Once	62	0/2
	16	Once	−15	0/3
Uracil, class IA	25	Daily × 5	85	1/3
	50	Daily × 5	166	3/3
	100	Daily × 5	200	2/3
	125	Once	107	1/3
	30.63	q3h × 5	137	1/3
	61.25	q3h × 8	37	0/3
	250	Once	−10	0/3
	500	Once	−60	0/3
Thymine, class IA	100	Daily × 5	124	1/3
	30.63	q3h × 7	156	2/3
	61.25	q3h × 7	103	1/3
5,6-Dihydrothymine, class IA	50	Daily × 5	157	2/3
	100	Daily × 5	0	0/3
	30.63	q3h × 8	75	1/3
	61.25	q3h × 6	121	2/3
1-Methyluracil, class IA	50	Daily × 5	88	1/3
	100	Daily × 5	19	0/3
	30.63	q3h × 8	34	0/3
	61.25	q3h × 8	110	1/3

*All experiments were ended arbitrarily at 200% ILS and any survivors were tabulated at that time. All therapy was started on the third day after tumor transplant; injection was ip. Untreated tumor controls died 10–11 days after transplant. In some instances efforts to reproduce above results have shown considerable variability. Data of R. J. Speer and H. Ridgway, Wadley Institutes of Molecular Medicine, Dallas, Tex.

on Day 10 and the kidneys were removed and prepared for histologic evaluation. Animals in control groups were either nontumored nontreated animals, or tumored nontreated animals. Multiple sections of each kidney were examined. A very brief summary of the results is compiled in table 9. In all cases the histopathologic changes were dose dependent and were found to affect only the proximal convoluted tubules, the other portions of the kidney remaining essentially normal. While the higher dose levels of *cis*-dichlorodiammineplatinum (II) caused generalized vacuolar (hydropic) degeneration of the proximal convoluted tubules, the higher doses of the "platinum-uracil blues" (class IA) produced mild degenerative changes generally, with some severe multiple small foci of necrosis.

Occasional foci of degenerative change or necrosis surrounded by considerably larger areas of histologically normal renal tissue would tend to indicate that the physiologic function of the kidney had not been severely altered. However, a generalized degenerative lesion affecting the proximal convoluted tubules of visible nephrons would be expected to result in more severe alterations in renal function. Therefore, we judge that the "platinum-uracil blues" (class IA) are less nephrotoxic than *cis*-dichlorodiammineplatinum (II) when compared at roughly equivalent therapeutic doses. To test these assertions, a study correlating the levels of blood urea nitrogen and creatinine with renal histopathologic changes is currently in progress.

TABLE 6.—Effects of "platinum blue" complexes on L1210 leukemia in BDF_1 mice*

Compound	Dose (mg/kg)	Schedule	%ILS	No. of survivors/ No. treated
Uracil, class IA	50	Daily × 5	16	0/3
	100	Daily × 5	26	0/3
	61.25	q3h × 8	30	0/3
	30.63	q3h × 8	0	0/3
	250	Once	27	0/3
	500	Once	-58	0/3
Thymine, class IA	100	Daily × 5	25	0/3
	30.63	q3h × 8	32	0/3
	61.25	q3h × 8	32	0/3
5,6-Dihydrothymine, class IA	50	Daily × 5	36	0/3
	100	Daily × 5	45	0/3
	30.63	q3h × 8	30	0/3
	61.25	q3h × 8	0	0/3
1-Methyluracil, class IA	50	Daily × 5	10	0/3
	100	Daily × 5	28	0/3
	30.63	q3h × 8	10	0/3
	61.25	q3h × 8	22	0/3

*All experiments were ended arbitrarily at 200% ILS and any survivors were tabulated at that time. All therapy was started on the third day after tumor transplant. Untreated tumor controls died 10–11 days after transplant. In some instances efforts to reproduce above results have shown considerable variability. Data of R. J. Speer and H. Ridgway, Wadley Institutes of Molecular Medicine, Dallas, Tex.

TABLE 7.—Effects of *cis*-dichlorodiammineplatinum (II) and "platinum blues" on ADJ/PC6A tumor in female C^- mice*

Compound	LD50 (mg/kg)	ID90 (mg/kg)	Therapeutic index
Cis-dichlorodiammineplatinum (II)†	13	1.6	8.1
Uracil, class IA‡	225	94	2.4
5,6-Dihydrothymine, class IA†	135	25	5.4
6-Chlorouracil, class IA†	200	190	1.05
5-Carbethoxyuracil, class IA†	670	250	2.7
1-Methyluracil, class IA†	670	50	13.4
5-Hydroxymethyluracil, class IA†	40	42	0.95

*Data of T. A. Connors, Chester Beatty Institute, London, England.
†Injections given as single ip doses starting 24 days after tumor implantation.
‡Injections given ip starting 24 days after tumor implantation, daily for 5 days.

APPENDIX

Physical Measurements

Most physical measurements were done on the class I "platinum-blue" antitumor complexes. These will be discussed along with some data available on the other classes. In general, infrared, electronic absorption, and proton magnetic resonance spectral data were collected.

It is difficult to interpret the infrared spectra of the class I compounds with regard to structural features. They are characterized by strong absorbances, from 3500 to 3000 cm^{-1} (NH_3 stretching region) and in the 1600–1250 cm^{-1} region, indicative of complexes having carbonyl and C = N groups. There are also a number of weak to medium bands in the region from 1150 to 500 cm^{-1} due to ring vibrations. About the only possible conclusions that can be drawn from these spectra are that the ammine ligands are present and a ring structure still exists for the pyrimidine ligand.

The electronic absorption spectra of the class IA py-

TABLE 8.—Effects of *cis*-dichlorodiammineplatinum (II) and "platinum-uracil blues" on survival times of mice bearing Ehrlich ascites tumor*

Compound	Dose (mg/kg)	Mean survival time (days ± SD)	%ILS (mean)
Control	—	8.3 ± 1.8	—
Cis-dichlorodiammine-platinum (II)	7	33.8 ± 16.9	307†
Uracil, class IA	100	24.7 ± 7.6	198
Uracil	200	25.2 ± 7.4	204
Uracil	300	36.0 ± 14.2	334
Control	—	10.1 ± 2.8	—
Cis-dichlorodiammine-platinum (II)	7	29.7 ± 4.2	194
Uracil, class IA	200	25.7 ± 4.5	154
Uracil	300	30.3 ± 6.3	200
Uracil	400	28.5 ± 4.2	182

*Six mice per group; each mouse received 10^7 ascites tumor cells on Day 0. Treatment was as a single ip injection on Day 1. Data of G. R. Gale, Veterans' Administration Hospital, Charleston, SC.

†Two animals in this group survived >60 days.

rimidine complexes contain broad absorption bands in the vicinity of 550–650 nm with molar extinction coefficients on the order of 500–1000 mol^{-1} cm^{-1}. The class IB complexes contain a single broad band near 720 nm of similar intensity. These blue and green compounds contain a very intense band near 290 nm indicating the presence of a pyrimidine moiety in these complexes.

The proton magnetic resonance spectra of the class IA uracil, thymine, and 5,6-dimethyluracil complexes were recorded on a Varian A56/60 spectrometer in D_2O solution. The uracil compound gave a spectrum containing peaks at 2.76 and 2.92 ppm (solvent at 4.47 ppm relative to DSS, water-soluble form of tetramethylsilane-TMS); the thymine compound spectrum contained a broad peak at 1.71 ppm and peaks at 2.77 and 2.93 ppm (solvent at 4.47 ppm). Both of these had weak peaks near 7.5 ppm, possibly due to unreacted free base as a contaminant. The spectrum of the 5,6-dimethyluracil compound contained peaks at 1.76 and 2.07 ppm relative to HDO at 4.47 ppm. From these spectra we can only conclude that the magnetic environment of the protons in the bases changes when they become coordinated in these blue complexes to increase the shielding of the protons. The spectrum of free uridine in D_2O contains doublets at 5.80 (H_5) and 7.65 (H_6) ppm; that of free thymine in dimethylformamide contains a sharp peak at 1.72 ppm (C_5-methyl protons) and a broad peak at 7.20 ppm (H_6) with TMS at 0.0 ppm.

We have had measurements done of the binding energy of the 4f electrons in platinum using X-ray photoelectron spectroscopy. For the uracil class I complex a value of 73.6 eV was obtained. As a comparison, the value for *cis*-dichlorodiammineplatinum (II) is 73.02 eV. Platinum (IV) complexes yield binding energies of 75.9–76.1 eV. Platinum (II) complexes have binding energies between 72.3 and 74.0 eV. This suggests that platinum in the class I uracil complex is divalent and seems to contradict any proposed structures requiring hydroxyl ions in the fifth and sixth coordination positions in a platinum (IV) complex (*see* the analysis section of this paper).

The infrared spectra of the class II blue complexes generally contain broad bands from 3600 to 3100 cm^{-1} (the O–H and N–H stretching region), a number of strong broad bands in the 1520–1270 cm^{-1} region (usually attributed to hydrogen bonding), and weaker bands from 1030 to 590 cm^{-1}. The class II compounds prepared from the class III products (*see* above) have similar spectra. These spectra have been discussed elsewhere (3) so we will not comment on them here. However, the class II acetamide purple complex has an infrared spectrum nearly identical to the blue complex except for a much more intense band at 3300 cm^{-1} (the N–H stretching region).

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TABLE 9.—Observed renal histopathologic changes in mice kidneys (limited to the proximal convoluted tubules)*

Compound	No. of injections (schedule)	Histopathologic changes				
		Doses (mg/kg)				
		0.5	1.0	2.0	3.0	7.0
Cis-dichlorodiammineplatinum (II)	8 (q3h)	Normal	Normal	—	—	—
	7 (daily × 7)	—	Normal	Mild degenerative changes (cloudy swelling)	Mild degenerative changes (cloudy swelling)	—
	1 (tumored animal)	—	—	—	—	Extensive degenerative changes (hydropic degeneration)
	1 (nontumored animal)	—	—	—	—	Extensive degenerative changes (hydropic degeneration)
		Doses (mg/kg)				
		50		100	150	400
Uracil, class IA	6 (q3h)	—		—	Focal areas of degeneration and necrosis; hyaline casts also present	—
	8 (q3h)	—		Focal areas of degeneration and necrosis; hyaline casts also present	—	—
	7 (daily × 7)	Mild congestion of renal cortex, otherwise normal		Extensive focal areas of degenerative changes (hydropic degeneration)	Isolated focal areas of degenerative changes (hydropic degeneration)	—
	1	—		—	—	Mild degenerative changes (cloudy swelling); cortical hyperemia

*— = Schedules and doses not tested.

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Development of Resistance to Rubidazone (NSC-164011) in Ehrlich Ascites Tumor In Vivo [1,2,3]

Torben Skovsgaard [4,5,6]

SUMMARY

Resistance to a new daunorubicin derivative, rubidazone, was developed in a strain of Ehrlich ascites tumor in vivo by treatment with the drug during 11 weekly passages of the tumor. In this tumor reciprocal cross resistance was found between rubidazone, daunorubicin, adriamycin, vincristine, and vinblastine. There was no change in sensitivity of the tumor to methotrexate, 6-mercaptopurine, BCNU, cytosine arabinoside, or actinomycin D. The resistance was stable for at least 71 weeks when treatment was continued and for at least 18 weeks when treatment was discontinued. Rubidazone given intraperitoneally was significantly less toxic than daunorubicin and adriamycin; unlike the findings with daunorubicin and adriamycin, late toxic death (after 30 days) often occurred. In the wild-type tumor the growth inhibition with rubidazone was less than with daunorubicin and adriamycin on an equal weight basis. The LD10 of rubidazone inhibited the growth to nearly the same extent as did the LD10 of daunorubicin and adriamycin.

[Cancer Chemother Rep 59:301–308, 1975]

A new semisynthetic derivative of daunorubicin, rubidazone, has been isolated by the Rhone-Poulenc Research Laboratory. Chemically, the drug belongs to the group of anthracyclines. The molecular structures of rubidazone (1),[7] daunorubicin (2), and adriamycin (3) are shown in figure 1.

Rubidazone has been demonstrated to exert a pronounced antineoplastic effect on several experimental tumors (1).[7] In a preliminary clinical report (4) the antileukemic activity of rubidazone proved to be on a level with that of daunorubicin (5) but the hematologic toxicity was less pronounced.

The value of anthracyclines in cancer chemotherapy is greatly limited by the rapid development of clinical drug resistance. An important cause is probably the emergence of a genetically stable drug-resistant population from an initially sensitive population of neoplastic cells.

[1] Received Apr 2, 1974; revised Aug 19, 1974; accepted Sept 11, 1974.

[2] Supported by the Anders Hasselbalch Foundation.

[3] Rubidazone was kindly supplied by the Rhone-Poulenc Research Laboratory, Paris, France. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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[5] The author is indebted to Severin Olesen Larsen for statistical assistance and Lis Hasholt for cytogenetic analysis. The technical assistance of Miss Inge Strarup, Miss Anette Jacobsen, and Mr. Jørgen Dusing is gratefully acknowledged.

[6] *Reprint requests to:* Torben Skovsgaard, MD, Department of Internal Medicine, Finsen Institute, 49 Strandboulevarden, DK 2100 Copenhagen, Denmark.

[7] Maral R, Julou L, Ganter P, et al. Benzoylhydrazone daunorubicine (22050 R.P.): toxicite, pharmacologie generale et activite antitumorale. Rhone Poulenc, unpublished report, 1972.

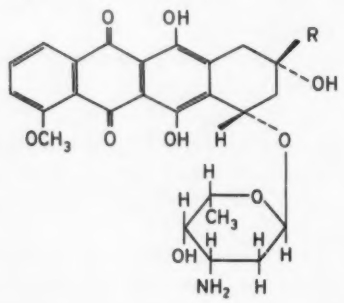
Clinical primary (inherent) resistance to rubidazone has already been described (4), and probably both primary and secondary (acquired) resistance will become a major problem, as they are in treatment with daunorubicin. For this reason, we studied the development of resistance to rubidazone in an experimental system.

Resistance has previously been developed to daunorubicin (6), adriamycin (7), vincristine (8), and vinblastine (8) in Ehrlich ascites tumor in vivo. In this biologic system reciprocal cross resistance between each of the four drugs was demonstrated.

In the present study, resistance to rubidazone was developed, the sensitivity pattern of the rubidazone-resistant tumor to other drugs was compared with that of the wild-type tumor, and the sensitivity to rubidazone of four sublines resistant to daunorubicin, adriamycin, vincristine, and vinblastine was investigated. Preliminary results have been reported (9).

MATERIALS AND METHODS

First-generation hybrids of female random-bred Swiss mice and male inbred DBA mice (DBA/2 CRT received from Texas Inbred Mice Co., Oct 1967) were used throughout the study. The treated and control groups consisted of eight to 18 mice weighing 17–21 g. Rubidazone



Rubidazone R = $C(CH_3)=N-NHOC-C_6H_5$

Daunomycin R = $COCH_3$

Adriamycin R = $COCH_2OH$

FIGURE 1.—Structures of the anthracyclines rubidazone, daunorubicin, and adriamycin.

was supplied as the hydrochloride. Daunorubicin and adriamycin were kindly supplied as hydrochlorides by Farmitalia Co., Milan, Italy. Vincristine and vinblastine, both supplied as sulfates, were obtained from Eli Lilly Co., Indianapolis, Indiana.

All drugs were administered intraperitoneally (ip) in isotonic solutions; the dosage was based on mouse weight. Rubidazone was dissolved in 0.9% NaCl, stored frozen at −20° C, and used within 3 weeks. Actinomycin D was dissolved in a 0.9% NaCl solution just before administration. The methods of administering the other drugs were as described previously (6,7).

In the toxicity experiments mice were given daily doses for 5 days and observed for 60 days (rubidazone) or 30 days (actinomycin D) after the first injection. The LD10, with 95% confidence limits, was calculated by a computerized program of probit analyses (6). Data for toxicity of daunorubicin, adriamycin, vincristine, vinblastine, cytosine arabinoside, BCNU, methotrexate, and 6-mercaptopurine were derived from previous experiments performed in our laboratory (6–8).

The wild-type tumor studied (EHR 2) was a hypotetraploid strain of Ehrlich ascites tumor obtained from Microbiological Associates, Inc., Bethesda, Maryland. Tumors were maintained in mice alternating in sex by weekly ip implantations with 0.2 ml of undiluted ascitic fluid.

Tumor growth was determined as follows: mice received implantations of 15×10^6 cells. Seven days after implantation the total volume of ascites tumor cells in each mouse was determined by removing the ascitic fluid, washing the peritoneal cavity, weighing the pooled ascitic fluid and washings, and determining the cell volume percent by centrifugation in a hematocrit centrifuge. When the ascitic fluid was blood stained, a correction was made on the basis of measured hemoglobin content. In experiments on tumors in which resistance was maintained by drug treatment, the tumor was left untreated during the week prior to the experiment.

Therapy experiments were performed after randomization of implanted mice. The drug was given by daily ip injections for 5 days

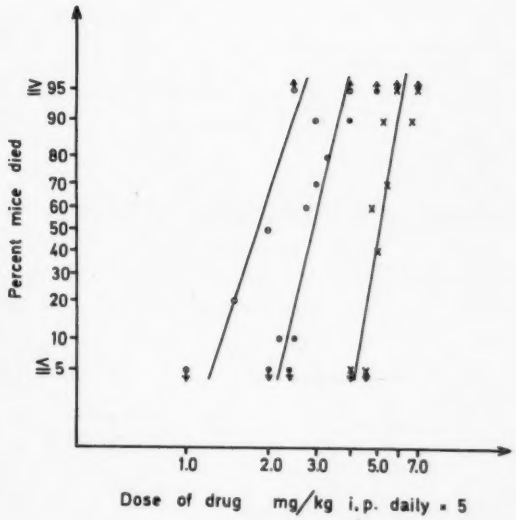


FIGURE 2.—Log-probit plot of toxicity of rubidazone (×————×) in female mice. LD10 was calculated as 4.4 mg/kg × 5 (95% confidence limits, 4.0–4.7 mg/kg). Ten mice were tested for each dose. Arrows pointing down and up indicate that either no mice or all mice in the group died. For comparison, data previously obtained in our laboratory for toxicity of daunorubicin (○————○) and adriamycin (●————●) are plotted (6,7).

(starting 24 hours after implantation), and the total volume of ascites tumor cells per mouse was determined 7 days after implantation. The mean total tumor cell volume in treated groups was calculated as a percent of the mean in a control group, together with the approximate standard error (SE) of this percentage. A "u" test was performed to evaluate the significance of differences in relative inhibition.

A hemocytometer was used for counting cells, and the mean single-cell volume was calculated as total ascites tumor cell volume per mouse divided by total ascites tumor cell number.

RESULTS

Toxicity

In female mice treated ip daily for 5 days, the LD10 for rubidazone was 4.4 mg/kg (range, 4.0–4.7 mg/kg),[8] compared with 1.3 mg/kg (range, 1.1–1.6 mg/kg)[8] for daunorubicin and 2.3 mg/kg (range, 2.2–2.4 mg/kg)[8] for adriamycin under identical conditions (6,7). A log-probit plot of the data for rubidazone and previous data obtained with daunorubicin and adriamycin is shown in figure 2. Unlike what has been described for daunorubicin and adriamycin (6,7), the mice treated with rubidazone often died after Day 30. For this reason an observation period of 60 days was chosen. Statistical evaluation showed that the LD10 for rubidazone differs highly significantly from the LD10 for adriamycin and daunorubicin ($P<0.001$).

[8] 95% Confidence limits.

Therapy for the Wild-Type Tumor

The response of the wild-type tumor (EHR 2) to rubidazone is shown as one of the curves in figure 3. The LD10 of rubidazone inhibited the growth to 14% of the control value. For direct comparison of the effects of rubidazone, daunorubicin, and adriamycin, the drugs were given to randomized groups of mice within the same experiment. The growth inhibition in this experiment is shown in table 1. When evaluated by the "student's t" test, treatment with rubidazone results in significantly less tumor inhibition ($P<0.01$) than treatment with daunorubicin and adriamycin on an equal weight basis. On the other hand, when the drugs were given in equitoxic doses (LD10),

TABLE 1.—Comparison between rubidazone, daunorubicin, and adriamycin of growth inhibition in wild-type Ehrlich ascites tumor

Drug	Dose (mg/kg ip daily × 5)	Total ascites tumor cell volume in treated mice* (% of control group)
Rubidazone	0.5	81 ± 10
Daunorubicin	0.5	44 ± 8
Adriamycin	0.5	44 ± 10
Rubidazone	1.0	41 ± 6
Daunorubicin	1.0	12 ± 4
Adriamycin	1.0	17 ± 5
Rubidazone	4.60	9 ± 2
Daunorubicin	1.25	8 ± 1
Adriamycin	2.10	4 ± 0.4

*Mean value ± SE. There were 8–10 mice in each test and control group.

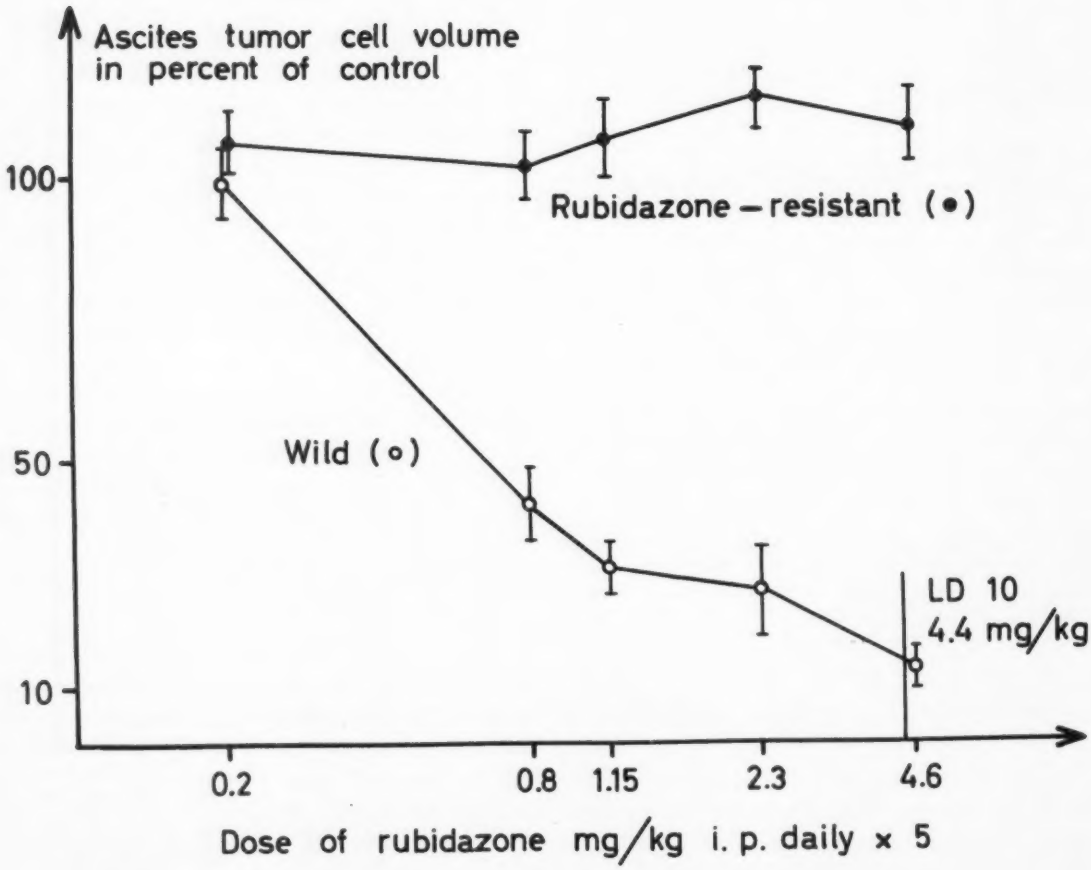


FIGURE 3.—Response to treatment with rubidazone of a wild-type Ehrlich ascites tumor and of a subline selected for resistance to rubidazone. Each dot represents the mean total ascites tumor cell volume 7 days after implantation with 15×10^6 cells in 8–12 treated mice (percent of mean total ascites tumor cell volume in 18 control mice). Vectors indicate standard errors of the mean.

nearly the same activity was obtained for rubidazone, daunorubicin, and adriamycin.

Development of Resistance to Rubidazone

A line of the wild-type tumor was maintained in mice by weekly implantation with 0.2 ml of undiluted ascitic fluid and treatment 5 days a week with rubidazone, starting with a dose of 1 mg/kg. After 11 passages during gradually increasing doses the tumor developed complete resistance to rubidazone at the LD10. One subline, EHR 2/RP+, was maintained with a dose of 4.6 mg/kg of rubidazone, while another subline, EHR 2/RP–, was maintained without treatment. Both lines (EHR 2/RP+ in the 71st passage and EHR 2/RP– in the 18th passage) have retained their resistance (*see* table 2).

Growth of the Rubidazone-Resistant Tumor

The growth of the untreated rubidazone-resistant tumor was determined in an experiment in which ten to 12 mice were implanted with 15×10^6 tumor cells. Seven days later the mean total cell volume and the mean ascites tumor cell number per mouse were determined as 1.32 ± 0.08 ml and $493 \times 10^6 \pm 28 \times 10^6$ cells respectively. These data do not

TABLE 2.—Response to rubidazone treatment of a wild-type Ehrlich ascites tumor, of a subline during and after development of resistance by treatment with rubidazone, and of sublines with resistance developed to daunorubicin, adriamycin, vincristine, and vinblastine

Tumor*	No. of weekly passages after first rubidazone treatment	Total ascites tumor cell volume in mice treated with rubidazone, 4.6 mg/kg ip daily × 5† (% of control group)
EHR 2		9 ± 2
EHR 2/RP+	11	106 ± 5
EHR 2/RP+	71	97 ± 9
EHR 2/RP−	33	86 ± 6
EHR 2/DM+		99 ± 9
EHR 2/AM+		96 ± 6
EHR 2/VCR+		95 ± 6
EHR 2/VBL+		95 ± 7

*EHR 2 = wild-type tumor; EHR 2/RP+ = subline selected for resistance to rubidazone; EHR 2/RP− = subline in which rubidazone treatment was discontinued after 15 wks; EHR 2/DM+, EHR 2/AM+, EHR 2/VCR+, and EHR 2/VBL+ = sublines selected for resistance to daunorubicin, adriamycin, vincristine, and vinblastine respectively.

†Mean value ± SE.

differ significantly from the data previously found (under identical conditions) with the wild-type tumor (6) and the subline resistant to vincristine (8). On the other hand, the sublines resistant to daunorubicin, adriamycin, and vinblastine grew to significantly smaller total cell volume as a result of a smaller total cell number as well as a smaller mean single-cell volume (6–8).

Cytogenetic Data

The karyotypic profile of the tumor line resistant to rubidazone was analyzed and compared with previous findings with the wild-type tumor (10). Both cell lines consisted of near-tetraploid cells with chromosome numbers around 77 and a fairly wide variation. Also, with respect to marker chromosomes (large metacentric chromosomes) both cell lines showed an identical pattern.

Response of the Rubidazone-Resistant Tumor

The response of the rubidazone-resistant tumor line to rubidazone is shown in figure 3. It appears that the tumor line is completely resistant to the drug. Table 3 shows the response of the rubidazone-resistant tumor line to various antileukemic drugs at doses equal to the LD10. The results are compared with the inhibition of the wild-type tumor under identical conditions. The rubidazone-resistant tumor was found to be cross resistant to daunorubicin, adriamycin, vincristine, and vinblastine. There was no change in sensitivity to cytosine arabinoside, BCNU, methotrexate, 6-mercaptopurine, or actinomycin D.

Response of Different Resistant Tumor Lines to Rubidazone

Table 2 shows the response to rubidazone treatment of the four tumor lines of Ehrlich ascites tumor in which resistance to daunorubicin, adriamycin, vincristine, and vinblastine had been developed. It appears that these tumor lines show complete cross resistance to rubidazone in a dose approximately equal to the LD10.

DISCUSSION

In this study development of resistance to a new daunorubicin derivative, rubidazone, was demonstrated in an Ehrlich ascites tumor in vivo. The resistance was stable for at least 71 weeks when treatment was continued and for at least 18 weeks when treatment was discontinued. The maintenance of resistance without treatment in the tumor resistant to rubidazone is not unexpected. The rate of growth for this tumor does not differ significantly from that of the wild-type tumor. In other words, a population of sensitive tumor cells which possibly might be present does not have any particular advantage. In a previous study (8) a corresponding observation was made for the tumor resistant to vincristine which also grows like the wild-type tumor. On the other hand, the tumors resistant to daunorubicin (6) and vinblastine (8), which have shown a rate of growth slower than the wild-type tumor, lost their resistance after discontinuation of treatment.

The tumor line selected for resistance to rubidazone showed cross resistance to daunorubicin, adriamycin, vincristine, and vinblastine. Tumor lines selected for resistance to

TABLE 3.—Growth inhibition by various cytotoxic drugs of a line of Ehrlich ascites tumor selected for resistance to rubidazone, compared with response of corresponding wild-type tumor

Drug	LD10* (mg/kg ip daily × 5)	Therapy: Dose (mg/kg ip daily × 5)	Therapy: Tumor cell volume in treated mice (% of control group): Wild-type Ehrlich ascites tumor†	Therapy: Tumor cell volume in treated mice (% of control group): Rubidazone-resistant Ehrlich ascites tumor	Response of rubidazone-resistant tumor compared with wild-type tumor‡
Rubidazone	4.4 (4.0–4.7)	4.6	9 ± 2	106 ± 5	R ($P<0.001$)
Daunorubicin	1.3 (1.1–1.6)	1.25	8 ± 1	100 ± 6	CR ($P<0.001$)
Adriamycin	2.3 (2.2–2.4)	2.1	4 ± 0.4	90 ± 5	CR ($P<0.001$)
Vincristine	0.33 (0.27–0.40)	0.30	64 ± 6	89 ± 6	CR ($P<0.01$)
Vinblastine	0.75 (0.65–0.89)	0.8	19 ± 3	69 ± 6	CR ($P<0.001$)
Cytosine arabinoside	200 (140–280)	200	48 ± 4	49 ± 6	NC
BCNU	14 (12–16)	15	56 ± 5	50 ± 2	NC
Methotrexate	1.0 (0.7–1.4)	1.0	15 ± 4	8 ± 4	NC
6-Mercaptopurine	120 (90–160)	100	85 ± 6	89 ± 9	NC
Actinomycin D	0.125 (0.089–0.170)	0.125	95 ± 7	91 ± 7	NC

*Nos. in parentheses indicate 95% confidence limits. LD10 is based on a 30-day observation period for all drugs except rubidazone and BCNU for which the observation period was 60 and 40 days respectively.

†Tumor cell volume in treated mice as percent of controls. Mean value ± SE.

‡Significance of differences in response evaluated by the "u" test. P value is mentioned only when the difference between the groups was significant. R = resistance; CR = cross resistance; NC = no change.

daunorubicin, adriamycin, vincristine, and vinblastine showed cross resistance to rubidazone.

Development of resistance, as shown here, to members of one class of chemotherapeutic agents resulting in cross resistance to members of another class of chemotherapeutic agents is of interest only in the event that it is not interpretable as a coincidence. As previously discussed (8), this probability is reduced if reciprocal cross resistance is demonstrated and if the sublines concerned originate from different cell clones. Considering also the previous findings (6–8), this study demonstrates reciprocal cross resistance between three anthracyclines and two vinca alkaloids. Several findings indicate that the tumor line selected for resistance to rubidazone also originated from a clone different from that of the tumor lines selected for resistance to daunorubicin, adriamycin, and vinblastine. In the first place, the rubidazone-resistant tumor line grows very rapidly, with a large mean single-cell volume (like the wild-type tumor but unlike the other three resistant sublines). Secondly, the rubidazone-resistant tumor line did not show a change in the sensitivity to cytosine arabinoside and BCNU as compared with the wild-type tumor. As previously described (6–8), the tumor lines resistant to daunorubicin, adriamycin, and vinblastine showed collateral sensitivity to these drugs. Finally, the tumor line resistant to rubidazone and the wild-type tumor showed common features with regard to karyotypic profile but different from the tumor lines resistant to daunorubicin, adriamycin, and vinblastine (10). Thus, cross resistance between anthracyclines and vinca alkaloids, demonstrated in this system, seems to reflect a general phenomenon rather than selection of a clone which by chance is resistant to anthracyclines as well as vinca alkaloids.

Cross resistance between vinca alkaloids and anthracyclines has also been described in another system; a strain of Chinese hamster cells

in which resistance to daunorubicin had been developed in vitro showed decreased sensitivity to vincristine (16).

The reciprocal cross resistance between the three anthracyclines is not surprising. This result is in accordance with previous findings in groups of compounds within which the structural formula differs only slightly, eg, vinca alkaloids (8,11), 4-amino-substituted folic acid analogs (12), purine analogs (13), pyrimidine analogs (14), and alkylating agents (15). Cross resistance in such cases is probably an expression of the common mechanism of action resulting in a common mechanism of resistance.

The fact that the tumor line resistant to rubidazone differs with respect to rate of growth (6,7) and cytogenetic pattern (10) from the tumor lines resistant to daunorubicin and adriamycin, suggests that these characteristics are not specifically related to the mechanism of resistance to the anthracyclines.

The mechanism of resistance to the anthracyclines has not been clarified. However, a decreased cellular drug uptake has proved to be an important factor with rubidazone (9) as well as with daunorubicin and adriamycin (17). For daunorubicin, it has been suggested that the underlying mechanism is an active outward transport of the drug (18).

LIST OF COMPOUNDS

Actinomycin D: NSC-3053

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6; daunomycin; rubidomycin (NSC-83142)

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid,*N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-

Rubidazone: NSC-164011; CAS reg. No. 36508-71-1; daunorubicin, benzoylhydrazone, monohydrochloride; RP 22050

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Antitumor Activity of Selected Amino Acid Derivatives Against Various Tumor Systems [1,2]

Koji Fukushima and Shigeshi Toyoshima [3,4]

SUMMARY

We have previously reported that from 350 amino acid (A-A) derivatives five were selected after the primary in vivo and in vitro screening tests. The five compounds which were found to possess potential antitumor activity against Ehrlich ascites carcinoma are as follows: β-naphthalene-sulfonyl-DL-tryptophan (A-91), β-naphthylaminomethyl-γ-aminobutyric acid (A-144), *N*-ethylcarbaminomethyl-L-isoleucine (A-145), *N*-9-fluorenylacetyl-L-phenylalanine (A-192), and *N*-propionyl-L-valine (A-195). The effect on life prolongation and tumor growth of these selected A-A derivatives against various types of tumors, including ascites and solid tumors in mice and ascites hepatomas in rats, was examined. A-A derivatives were administered once daily for 3 consecutive days starting 24 hours after tumor implantation. Experimental results showed that among the five A-A derivatives possessing considerable activity against Ehrlich carcinoma, A-144 and A-145 were found to be more effective than chromomycin A_3 and showed activity similar to that of cyclophosphamide against ascites Sarcoma 180. A-A derivatives showed slight antitumor activity against SR61 and L1210 leukemias. In rat ascites hepatoma, such as AH13, AH7974, AH60C, and Yoshida sarcoma, only A-145 showed a significant prolongation of the lifespan in the control groups. The five selected A-A derivatives significantly inhibited the growth of Nakahara-Fukuoka sarcoma and solid Sarcoma 180. These findings indicate that among the five A-A derivatives, A-145 appeared to be the most active against ascites and solid tumors.

[Cancer Chemother Rep 59:309–318, 1975]

In previous reports, five amino acid (A-A) derivatives (fig 1), selected from the first screening tests of 350 compounds, were found to be highly effective against Ehrlich ascites carcinoma in vivo. Some of these selected A-A derivatives also had an inhibiting effect on macromolecular synthesis in tumor cells (1).

Several studies have been carried out on the effects of A-A analogs on tumor growth (2–7).[5]

[1] Received May 24, 1974; revised Sept 5, 1974; accepted Sept 24, 1974.

[2] Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text. A-91, A-144, A-145, A-192, and A-195 were obtained from the Central Research Laboratories, Ajinomoto Company, Tokyo, Japan.

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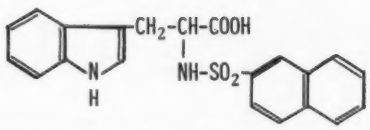
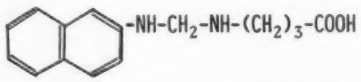
[5] Carter SK. Chemotherapy fact sheet: 1-aminocyclopentanecarboxylic acid: a review, July 1970. Prepared by the National Cancer Institute, Bethesda, Md.



N-β-naphthalene-sulfonyl-DL-tryptophan(A-91)



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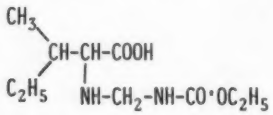
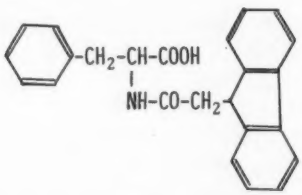
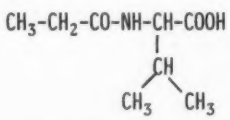


N-ethylcarbaminomethyl-L-isoleucine(A-145)



N-9-fluorenylacetyl-L-phenylalanine(A-192)



N-propionyl-L-valine(A-195)

FIGURE 1.—Structures of the five A-A derivatives.

After the introduction of aminopterin, a series of antifolic analogs and a pteroylglutamic acid analog were synthesized to provide agents with greater activity and a broader therapeutic spectrum for the treatment and control of neoplastic diseases (Delmonte et al [8]; Burchenal et al [9]). A series of diazoacetyl derivatives of glycine and glycyl-glycine were synthesized and their antitumor properties were investigated by Baldini et al (10). Of these, diazoacetyl-glycine amide proved to be one of the most active against rodent tumors. DeWys et al (11) have reported that mimosine, an alpha amino acid, showed antitumor activity in the Walker 256 carcinosarcoma system. Mimosine appears to act as a tyrosine inhibitor (12) and thus may be classed with L-asparaginase, the glutamic acid antagonists, 2-thiophenealanine, desoxypyridoxine, and the other agents affecting A-A metabolism (13).

The present studies were carried out to determine whether the antitumor activity of selected A-A derivatives is unique to the Ehrlich carcinoma or can also be seen in other tumor systems.

MATERIALS AND METHODS

Male ddY and BDF_1 mice, weighing 15–20 g, and male Donryu rats, weighing 150–200 g, were used. The effect on life prolongation and tumor growth was determined from experiments conducted on the following tumors: Ehrlich ascites carcinoma (ddY mice), ascites and solid Sarcoma 180 (ddY mice), SR61 leukemia (ddY mice) (Shirasu et al [14]), L1210 leukemia (BDF_1 mice), Nakahara-Fukuoka (N-F) sarcoma (ddY mice), rat ascites hepatoma (such as AH13, AH7974, AH60C), and Yoshida sarcoma (Donryu rats).

Tumor cells were suspended in sterile phosphate buffered saline, and 1×10^7 cells of SR61 leukemia, 1×10^6 cells of L1210 leukemia, or 5×10^6 cells of Ehrlich ascites carcinoma, ascites Sarcoma 180, AH13, AH7974, AH60C, and Yoshida sarcoma were implanted intraperitoneally (ip). A total of 50 mg of N-F sarcoma fragments or 100 mg of solid Sarcoma 180 fragments was implanted subcutaneously (sc).

The five A-A derivatives were suspended in 0.1% carboxymethylcellulose as vehicle and were administered at a volume of 0.01 ml/g of body weight. They were administered ip or orally once daily for 3 consecutive days at doses of one fifth of the LD50 beginning 24 hours after tumor implantation. In the L1210 leukemia system, A-A derivatives were administered ip once daily on 5 consecutive days beginning 24 hours after tumor implantation.

The LD50 of the A-A derivatives was calculated by giving a single injection and determining the number of survivors on the 14th day

after the injection by the method of Litchfield and Wilcoxon (15). Antitumor activity was determined by comparing the mean survival time and the average tumor weight of the treated groups with those of the control groups. These parameters were measured on the 15th day after implantation of N-F sarcoma and on the 21st day after implantation of solid Sarcoma 180. The percent increases in lifespan (%ILS) and the inhibition of tumor growth were calculated from the mean survival time and the average tumor weight respectively. Animals were weighed on Days 0, 1, and 10, and the average weight changes were determined.

RESULTS

To reconfirm the potential antitumor activity of the five A-A derivatives against Ehrlich ascites carcinoma, the effects of the drugs administered ip or orally daily (Days 1–3) on the survival of mice bearing Ehrlich ascites carcinoma were examined (table 1). As shown in this table, A-192 was more effective when administered orally than mitomycin C and bleomycin (employed as standard reference drugs). A-91, A-145, and A-195 significantly prolonged the lifespan of mice bearing Ehrlich ascites carcinoma (37.6%, 66.7%, and 64.0% ILS respectively), while A-144 showed no significant prolongation. Some of the five A-A derivatives, possessing remarkable antitumor activity when administered ip, were found to be effective against Ehrlich ascites carcinoma even when administered orally.

The effects of the five A-A derivatives administered ip daily (Days 1–3) on the survival time of mice bearing ascites Sarcoma 180 are shown in table 2. A-144 and A-145 were more effective than chromomycin A_3; the two compounds showed activity similar to that of cyclophosphamide against ascites Sarcoma 180.

The effects of the five A-A derivatives administered ip daily (Days 1–3) on the survival time of mice bearing SR61 leukemia are shown in table 3. No A-A derivatives were as effective as mitomycin C. However, A-91 and A-192 showed activity similar to that of 6-mercaptopurine riboside.

The antitumor activity of the five A-A derivatives against L1210 leukemia was examined. A-A derivatives were administered ip once daily for 5 consecutive days at doses of one fifth of the LD50 beginning 24 hours after tumor implantation. A-145 showed slight antitumor activity (36% ILS) against L1210 leukemia, but the other A-A derivatives produced negative or equivocal results.

The effects of the five A-A derivatives on the growth of N-F sarcoma and solid Sarcoma 180 were further examined (tables 4 and 5). Antitumor activity of these compounds was evaluated according to tumor weight on the 15th day after implantation for N-F sarcoma and on the 21st day for solid Sarcoma 180. At daily doses of one fifth of the LD50 of cyclophosphamide and A-195, the compounds resulted in a relatively higher toxicity, causing weight loss and early death. In mice with N-F sarcoma, the daily dosage was reduced to one tenth of the LD50. As shown in table 4, the five A-A derivatives were especially active against N-F sarcoma and were more effective than mitomycin C and chromomycin A_3. These compounds also showed similar or greater inhibition of tumor growth than that observed with cyclophosphamide (43.2 mg/kg/day).

The effects of the five A-A derivatives on the tumor growth of mice bearing solid Sarcoma 180 are shown in table 5. With the exception of A-192, the A-A derivatives showed significant growth inhibition of more than 70% greater than that of mitomycin C.

Thus, it is considered that these five A-A derivatives are more effective against both ascites and solid tumors in mice.

Figures 2–5 give a summary of the results of experiments in which A-A derivatives were administered ip for 3 days after tumor implantation to rats bearing three strains of ascites hepatoma and Yoshida sarcoma. It has been reported (16) that, although AH13 was sensitive to alkylating agents, AH7974 was rather insensitive. With the exception of A-91, the A-A derivatives significantly prolonged the lifespan of rats bearing AH13 compared to tumor controls (fig 2). Although A-145 was less active than mitomycin C, it was more active against AH13 than the other A-A derivatives when treatment was administered ip. Moreover, A-145 significantly prolonged the

TABLE 1.—Effect of A-A derivatives on survival time of mice bearing Ehrlich ascites carcinoma

Compounds	Dose* (mg/kg)	Mean survival time† (days ± SD)	No. of survivors at 60th day/ No. treated	%ILS
None (tumor control)	—	18.9 ± 1.4	0/10	—
Mitomycin C	1.5 (ip)	47.0 ± 19.1‡	3/10	147.9
	13 (oral)	19.5 ± 8.6	0/10	3.1
Bleomycin	0.5 (ip)	44.9 ± 17.0‡	3/10	136.3
	30 (oral)	33.1 ± 11.4‡	1/9	75.1
A-91	170 (ip)	51.2 ± 12.1‡	6/10	169.5
	1000 (oral)	26.0 ± 4.0‡	0/8	37.6
A-144	210 (ip)	56.0 ± 9.0‡	11/14	194.7
	1000 (oral)	20.8 ± 4.4	0/10	10.1
A-145	300 (ip)	58.8 ± 6.7‡	8/10	209.5
	1000 (oral)	31.5 ± 2.7‡	0/8	66.7
A-192	35 (ip)	56.5 ± 5.6‡	9/13	197.4
	370 (oral)	39.7 ±16.0‡	3/9	110.1
A-195	300 (ip)	55.2 ± 7.5‡	7/10	190.4
	1000 (oral)	31.0 ± 3.1‡	0/8	64.0

*Administered once daily for 3 consecutive days beginning 24 hours after tumor implantation.

†Statistical analysis compared mean survival time of treated animals with that of controls using the "student's t" test.

‡$P < 0.01$.

TABLE 2.—Effect of A-A derivatives on survival time of mice bearing ascites Sarcoma 180

Compounds	Dose* (mg/kg, ip)	Mean survival time† (days ± SD)	No. of survivors at 60th day/ No. treated	%ILS
None (tumor control)	—	15.4 ± 2.2	0/10	—
Mitomycin C	1.5	41.3 ± 16.2‡	2/8	168.2
Cyclophosphamide	86.4	30.9 ± 11.4‡	1/10	100.7
Chromomycin A_3	0.4	24.1 ± 7.5‡	0/10	56.5
A-91	170	17.1 ± 5.3	0/10	11.0
A-144	210	30.9 ± 17.1§	2/10	100.7
A-145	300	29.4 ± 12.4‡	1/10	90.9
A-192	35	15.4 ± 2.7	0/10	0
A-195	150	22.6 ±5.4‡	0/10	46.8

*Administered once daily for 3 consecutive days beginning 24 hours after tumor implantation.

†Statistical analysis compared mean survival time of treated animals with that of controls using the "student's t" test.

‡$P < 0.01$.

§$P < 0.05$.

TABLE 3.—Effect of A-A derivatives on survival time of mice bearing SR61 leukemia

Compounds	Dose* (mg/kg, ip)	Average weight change† (%)	Mean survival time‡ (days ± SD)	Survivors§	%ILS
None (tumor control)	—	+7.4	4.8 ± 0.8	0/20	—
Mitomycin C	1.5	+3.4	15.6 ± 5.3¶	20/20	225.0
6-Mercaptopurine riboside	60	−2.2	7.8 ± 4.3¶	10/20	62.5
A-91	170	−3.7	7.5 ± 3.8¶	12/20	56.3
A-144	210	−5.7	7.2 ± 1.7¶	8/20	46.9
A-145	300	−5.4	5.7 ± 0.9**	2/10	16.3
A-192	35	−1.5	7.5 ± 5.4**	3/10	56.3
A-195	300	−4.3	5.8 ± 1.4¶	3/10	20.9

*Administered once daily ip for 3 consecutive days beginning 24 hours after tumor implantation.

†Average weight change from start to end of drug treatment.

‡Statistical analysis compared mean survival time of treated animals with that of controls using the "student's t" test.

§Survivors at the time of death of the controls.

¶$P < 0.01$.

**$P < 0.05$.

TABLE 4.—Effect of A-A derivatives on tumor growth of mice bearing N-F sarcoma

Compounds	Dose* (mg/kg, ip)	Average weight change† (g ± SD)	Average tumor weight‡ (mg ± SD)	Inhibition of tumor growth (%)
None (tumor control)	—	3.7 ± 2.1	2494 ± 683	—
Mitomycin C	1.5	3.0 ± 0.6	1221 ± 734§	51.1
Cyclophospha-mide	86.4	(−)3.6 ± 1.4	211 ± 98§	91.5
	43.2	1.7 ± 0.6	835 ± 728§	66.5
Chromomycin A_3	0.4	3.4 ± 3.1	1208 ± 602§	51.6
A-91	170	2.9 ± 1.5	819 ± 541§	67.2
A-144	210	3.6 ± 2.3	843 ± 807§	66.2
A-145	300	1.5 ± 1.2	757 ± 425§	69.7
A-192	35	3.3 ± 1.6	594 ± 588§	76.2
A-195	150	2.5 ± 1.3	598 ± 362§	76.0

*Administered once daily for 3 consecutive days beginning 24 hours after tumor implantation.

†Average weight change from start of treatment to Day 15.

‡Average tumor weight as measured on Day 15.

§Significant difference from controls ($P < 0.01$).

TABLE 5.—Effect of A-A derivatives on tumor growth of mice bearing solid Sarcoma 180

Compounds	Dose* (mg/kg, ip)	Average weight change† (g ± SD)	Average tumor weight‡ (mg ± SD)	Inhibition of tumor growth (%)
None (tumor control)	—	5.3 ± 3.1	2887 ± 548	—
Mitomycin C	1.5	2.5 ± 1.8	817 ± 365§	71.7
A-91	170	3.2 ± 1.8	459 ± 321§	84.1
A-144	210	4.1 ± 1.4	523 ± 151§	81.9
A-145	300	3.7 ± 1.5	512 ± 281§	82.3
A-192	35	4.1 ± 2.1	1140 ± 898§	60.5
A-195	300	4.5 ± 0.9	725 ± 468§	74.9

*Administered ip once daily for 3 consecutive days beginning 24 hours after tumor implantation.

†Average weight change from start of treatment to Day 21.

‡Average tumor weight as measured on Day 21.

§Significant difference from controls ($P < 0.01$).

lifespan of rats bearing AH7974, AH60C, and Yoshida sarcoma but was less effective than mitomycin C (figs 3–5). On the 13th day after termination of A-145 treatment in rats bearing AH60C, the tumor cells grew again, culminating in death from invasion into various tissues. However, in the AH7974 and Yoshida sarcoma groups given 300 mg/kg ip, three animals survived for 90 days. Observations during this period showed that no tumor cells were present in the ascites (figs 3 and 5). A-91 and A-195 resulted in more toxicity to rats bearing AH7974 or AH60C when a daily dose of one fifth of the LD50 was administered, resulting in weight losses and early deaths.

Observations of the antitumor effect of the five A-A derivatives on rats bearing AH13, AH7974, AH60C, and Yoshida sarcoma indicated that only A-145 was somewhat effective against rat ascites hepatoma.

DISCUSSION

Five of approximately 350 A-A derivatives were found to have antitumor activity in vivo and in vitro from the primary screenings (1). The five A-A derivatives were also capable of inhibiting the tumor growth of Ehrlich ascites carcinoma on the 10th day after tumor implantation. Antitumor activity of the five A-A derivatives, when administered ip once daily for 3 or 5 consecutive days against various types of tumors (both mouse ascites and solid tumors and rat ascites hepatomas), is summarized in table 6. The varying degrees of antitumor effects were observed as a function of the tumor systems used. In mouse ascites tumors, the five A-A derivatives were found to be more active against Ehrlich ascites carcinoma than Sarcoma 180, SR61 leukemia, and L1210 leukemia (table 6). Moreover, A-145, A-192, and A-195 were also more effective whether administered ip or orally (table 1). Although only A-145 showed slight antitumor activity against L1210 leukemia, the other A-A derivatives produced negative or equivocal results. These findings suggest that the Ehrlich carcinoma may be more sensitive to metabolic insults than the other tumors studied. The diverse effects of A-A derivatives against various tumors may be due to the difference in ability to transport these compounds into tumor cells.

There are suggestive results indicating that uptake of ^{14}C-labeled A-145 by Ehrlich ascites carcinoma was significantly higher than that of ^{14}C-A-145 by ascites Sarcoma 180 for 30 minutes after the ^{14}C-labeled compound was injected ip (unpublished observations).

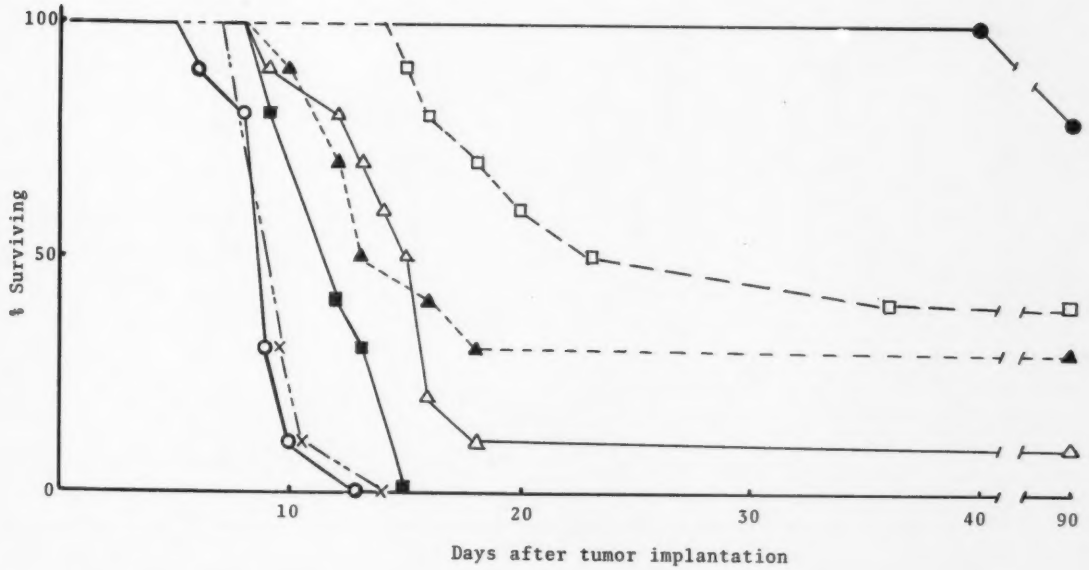


FIGURE 2.—Effect of selected A-A derivatives on survival time in rats bearing ascites hepatoma AH13. A-A derivatives were given ip for 3 consecutive days to rats and 5×10^6 AH13 cells were inoculated ip. ○ = Tumor control; × = A-91 (170 mg/kg); △ = A-144 (210 mg/kg); □ = A-145 (300 mg/kg); ▲ = A-192 (35 mg/kg); ■ = A-195 (300 mg/kg); ● = mitomycin C (0.5 mg/kg).

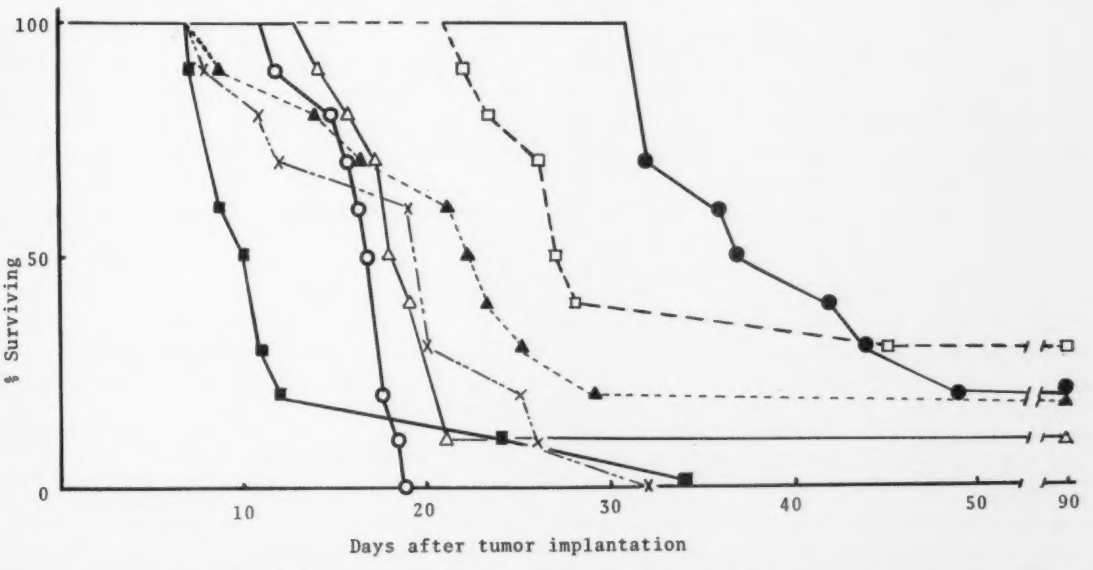


FIGURE 3.—Effect of selected A-A derivatives on survival time in rats bearing ascites hepatoma AH7974. A-A derivatives were given ip for 3 consecutive days to rats and 5×10^6 AH7974 cells were inoculated ip. ○ = Tumor control; × = A-91 (170 mg/kg); △ = A-144 (210 mg/kg); □ = A-145 (300 mg/kg); ▲ = A-192 (35 mg/kg); ■ = A-195 (300 mg/kg); ● = mitomycin C (0.5 mg/kg).

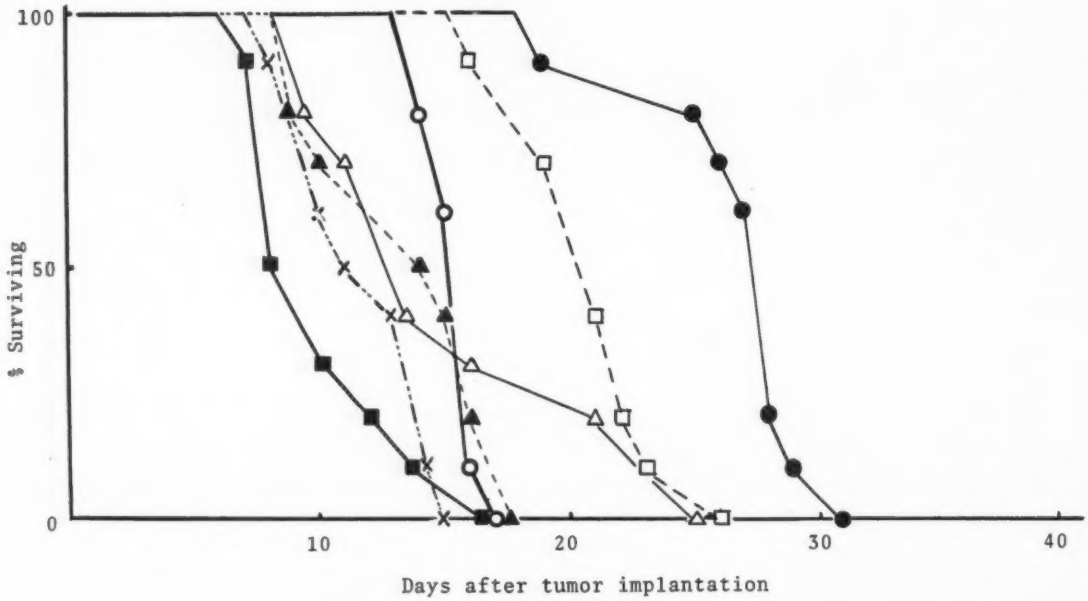


FIGURE 4.—Effect of selected A-A derivatives on survival time in rats bearing ascites hepatoma AH60C. A-A derivatives were given ip for 3 consecutive days to rats and 5×10^6 AH60C cells were inoculated ip. ○ = Tumor control; × = A-91 (70 mg/kg); △ = A-144 (210 mg/kg); □ = A-145 (300 mg/kg); ▲ = A-192 (35 mg/kg); ■ = A-195 (300 mg/kg); ● = mitomycin C (0.5 mg/kg).

In mouse solid tumors, the five A-A derivatives were found to possess remarkable antitumor activity against both N-F sarcoma and solid Sarcoma 180 (table 6), and additional experiments, using the macroautoradiography technique, showed that ^{14}C-labeled A-A derivatives were incorporated into solid tumors in mice (17). A single dose of 300 mg/kg of A-195 administered to mice bearing N-F sarcoma resulted in progressive weight loss and early death; similar results were also observed in the cyclophosphamide group (86.4 mg/kg/day).

In rat ascites hepatoma, the most favorable antitumor effects of A-A derivatives were observed in AH13. Results of AH7974, AH60C, and Yoshida sarcoma tests were less favorable. A similar response was observed in a study of the effect of alkylating agents (16). This suggests that AH13 may be more sensitive to metabolic insults than the other ascites hepatomas observed.

Overall results indicate that A-145 was most active against various types of tumors with the exception of mouse leukemia (table 6). In addition, A-145 has a remarkable inhibiting effect on DNA, RNA, and protein synthesis in tumor cells (1). Although little is known about the structural requirements for in vivo antitumor activity of A-A derivatives, the limited data presented here offer some suggestions regarding the aminomethyl substituent (–NH–CH_2–NH–) in A-144 and A-145 as the active group against rodent tumors.

Further research and attempts are presently being made to determine unequivocally the primary biochemical locus of action of the five A-A derivatives.

LIST OF COMPOUNDS

A-91: *N*-β-naphthalene-sulfonyl-DL-tryptophan
A-144: β-naphthylaminomethyl-γ-aminobutyric acid
A-145: *N*-ethylcarbaminomethyl-L-isoleucine
A-192: *N*-9-fluorenylacetyl-L-phenylalanine
A-195: *N*-propionyl-L-valine
Aminopterin: NSC-739; CAS reg. No. 54-62-6; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl]methyl]amino)benzoyl]-

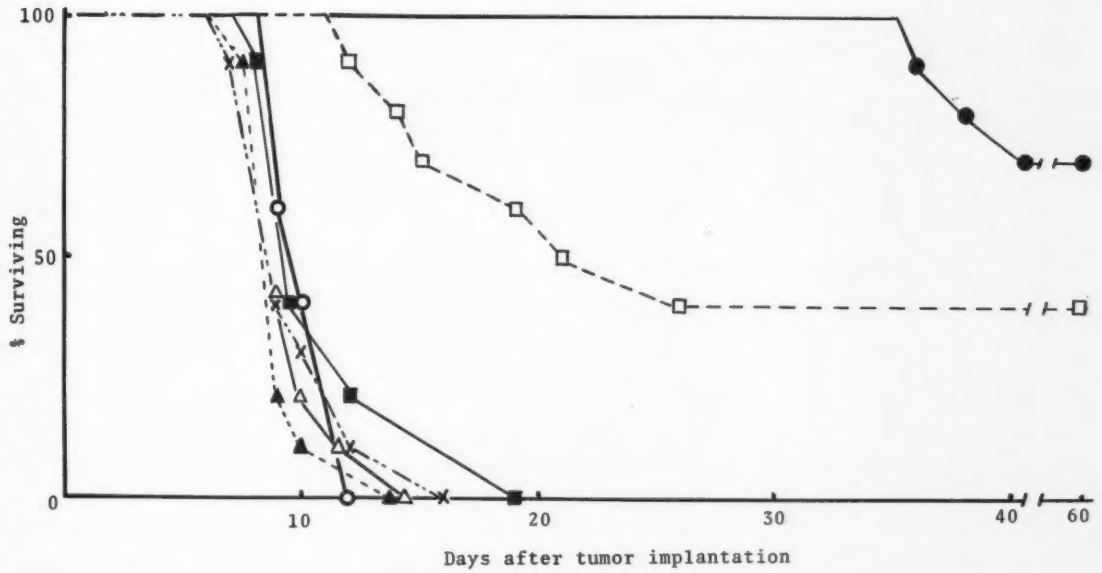


FIGURE 5.—Effect of selected A-A derivatives on survival time of rats bearing Yoshida sarcoma. A-A derivatives were given ip for 3 consecutive days to rats and 5×10^6 Yoshida sarcoma cells were inoculated ip. ○ = Tumor control; × = A-91 (170 mg/kg); △ = A-144 (210 mg/kg); □ = A-145 (300 mg/kg); ▲ = A-192 (35 mg/kg); ■ = A-195 (300 mg/kg); ● = mitomycin C (0.5 mg/kg).

L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
Bleomycin: NSC-125066
Chromomycin A_3: NSC-58514; CAS reg. No. 7059-24-7
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate
Desoxypyridoxine: NSC-3063; CAS reg. No. 148-51-6; 3-pyridinemethanol, 5-hydroxy-4,6-dimethyl-, hydrochloride
6-Mercaptopurine riboside: NSC-4911; CAS reg. No. 574-25-4; 9*H*-purine-6-thiol, 9-β-D-ribofuranosyl-; 6-MPR
Mimosine: NSC-69188; CAS reg. No. 500-44-7; 1(4*H*)-pyridinealanine, 3-hydroxy-4-oxo-
Mitomycin C: NSC-26980; CAS reg. No. 50-07-7
2-Thiophenealanine: NSC-754; CAS reg. No. 139-86-6; 2-thienylalanine

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TABLE 6.—Summarized data on antitumor activity of A-A derivatives in a variety of animal tumors

Tumors	Antitumor activity†				
	A-91* (170 mg/kg)	A-144* (210 mg/kg)	A-145* (300 mg/kg)	A-192* (35 mg/kg)	A-195* (300 mg/kg)
Ehrlich ascites carcinoma	+++	+++	+++	+++	+++
Ascites Sarcoma 180	-	++	+	-	-
SR61 leukemia	+	+	-	+	-
L1210 leukemia	-	-	+	-	-
Solid Sarcoma 180	+++	+++	+++	++	++
N-F sarcoma	++	++	++	++	++
AH13	-	+	+++	+	-
AH7974	-	-	+	-	-
AH60C	-	-	+	-	-
Yoshida sarcoma	-	-	+++	-	-

*Administered ip once daily for 3 consecutive days beginning 24 hours after tumor implantation. In L1210 leukemia, A-A derivatives were administered ip once daily for 5 consecutive days beginning 24 hours after tumor implantation.

†Tumor weight ratio (T/C) for the solid tumors and %ILS for the ascites tumors. For Ehrlich ascites carcinoma, ascites Sarcoma 180, and rat ascites hepatoma: - = <50% ILS; + = >50% but <100% ILS; ++ = >100% but <150% ILS; +++ = >150% ILS. For SR61 and L1210 leukemias: - = <25% ILS; + = >25% ILS. For solid Sarcoma 180 and N-F sarcoma: - = >0.6 T/C; + = >0.4 but <0.6 T/C; ++ = >0.2 but <0.4 T/C; +++ = >0 but <0.2 T/C.

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Concanavalin A (NSC-143504): Its Action on Experimental Tumor Cells and Possible Use in Cancer Chemotherapy[1,2]

H. Lin,[3,4] W. R. Bruce,[3] and M. J. Walcroft [3,5,6]

SUMMARY

Concanavalin A administered to mice kills KHT fibrosarcoma tumor cells and, less markedly, AKR lymphoma cells at doses where it has no effect on numbers of normal hematopoietic colony-forming cells. Concanavalin A in vitro agglutinates suspensions of cells prepared from murine and human tumors but does not agglutinate suspensions prepared from most normal mouse tissues. In spite of its selective action against tumor cells at the cellular level, the possibilities for using concanavalin A therapeutically are limited by its unusual toxicity, a generalized vasculitis that is seen in the mouse, monkey, and pig at approximately the same dose level.

[Cancer Chemother Rep 59:319–326, 1975]

Concanavalin A, a lectin derived from the Jackbean (*Canavalia ensiformis*), has a remarkable selectivity. It forms complexes and induces precipitation of polysaccharides, glycolipids, and glycoproteins with α-D-mannopyranosyl, α-D-glucopyranosyl, or β-D-fructofuranosyl residues at their nonreducing chain ends (1–4). It agglutinates the red blood cells of some species, ie, the mouse or dog, at concentrations where it has no effect on the red blood cells of others, ie, the pig or man (5). In addition, it agglutinates and kills transformed cells in vitro under conditions where it has no such effect on normal cells (6–8).

Our interest in concanavalin A relates to the possible use of this selectivity in cancer chemotherapy. We have therefore measured the sensitivity of two types of experimental tumor cells and hematopoietic stem cells to concanavalin A administered in vivo to mice. The tumor lines used are typical of transplanted leukemia and solid tumors. The hematopoietic colony-forming stem cells are normal cells, usually severely affected by cancer chemotherapeutic drugs (9–10). The striking selectivity which concanavalin A displays in killing tumor cells without affecting the normal cells prompted us to explore the possibility of using the drug via systemic administration as a potential cancer chemotherapeutic agent in man.

MATERIALS AND METHODS

Chemicals

Concanavalin A was obtained from two sources. A carbohydrate-free 2X crystallized preparation (supplied by Nutritional Biochemical Corp., Cleveland, Ohio) was used for the

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in vitro and mouse experiments. It was kept in saturated sodium chloride solution and diluted to the desired concentration just before use. Concanavalin A prepared by the method of Goldstein (a gift from Calbiochem, San Diego, Calif) was used in large animals after confirming that its effects in vitro and in mice were the same as those of the other preparation. Material injected into pigs and monkeys was passed through activated charcoal (No. 60 "Supercarbac," supplied by Cellulo Co., Hoboken, NJ) to remove pyrogens and was then sterilized by ultrafiltration. Concentration was assessed by optical density at 280 mμ (1).

Animals

AKR/J and C3H/HeJ mice (6–8 weeks old) weighing 18–22 g were obtained from the Jackson Laboratory, Bar Harbor, Maine. The pigs were of hybrid stock (Hampshire, Lacombe and Duroc of Connaught Medical Research Laboratories). They were 10–12 weeks of age and weighed 13–17 kg when first used. Cynomolgus monkeys (*Macaca fascicularis*), 2–3 kg and 16 months of age, were obtained from the Philippines through the Connaught Medical Research Laboratories. Baboon and chimpanzee blood was obtained from the Primate Laboratory of the National Cancer Institute, Bethesda, Maryland.

Cells and Transplantation Assays

The number of normal hematopoietic colony-forming units (CFU) was determined by the method of Till and McCulloch (11). In brief, bone marrow cell suspensions were prepared from the femurs of normal and concanavalin A-treated young AKR or C3H mice. Appropriate dilutions were made and the cell suspensions were injected intravenously (iv) into groups of supralethally irradiated (950 rads) syngeneic mice. Ten days later the recipient mice were killed and their spleens were removed and placed in Bouin's fixative. The number of macroscopic colonies on the surface of the spleens was counted and the results were reported as a fraction of the CFU per femur for the control untreated mice.

The transplanted AKR lymphoma line used in these experiments arose from a spontaneous AKR lymphoma and was carried in serial passage by the iv injection of lymphoma cells prepared from the spleens. Cells from transfer Nos. 220–240 were used. The proliferative capacity of tumor cells was measured by the method described by Bruce et al (10). Briefly, cells were flushed out from the bone marrow of femurs with medium CMRL 1066 and a fraction of the cell suspension was injected iv into a group of 20 syngeneic mice. These mice were killed 8 days later and the number of macroscopic surface colonies on the spleen was counted. Results were reported as a fraction of CFU per femur for the control lymphoma-bearing mice.

Transplanted KHT sarcoma was obtained from R. S. Bush (Ontario Cancer Institute, Toronto, Canada) at the 110th passage. This tumor arose spontaneously in a C3H mouse and was isolated by Kallman et al (12). The line was transplanted in this laboratory by injecting 1×10^5 cells subcutaneously (sc) into the flanks of female C3H mice, passages 115–140 being used for these studies. For most of the experiments, tumors 8–10 mm in diameter were used. The number of tumor cells capable of forming macroscopic colonies in the lung was measured by the lung colony method described by Hill and Bush (13). In brief, the tumor was excised and minced with scissors, and the mince was pressed through a coarse stainless steel screen (80 mesh/inch) with phosphate buffered saline. The cell suspension was then incubated with 0.05% trypsin and 0.02 mg/ml of DNAse at 37°C for 25 minutes, centrifuged at 1000 rpm for 10 minutes, and washed twice with medium CMRL 1066. Appropriate cell suspensions were made in medium containing 6×10^6 heavily irradiated tumor cells/ml (10,000 rads of ^{137}Cs γ-radiation) and injected in a volume of 0.5 ml into the tail veins of groups of syngeneic mice. The heavily irradiated cells were added to increase the colony efficiency (13). After 17 days, these recipient mice were killed and their lungs were removed and placed in Bouin's fixative. The number of macroscopic surface colonies was counted and results were reported as the fraction of the CFU per tumor for the control untreated mice.

The transplanted plasma cell tumor was the

PC-5 line obtained from D. E. Bergsagel (Ontario Cancer Institute, Toronto, Canada). The Lewis lung tumor was obtained from J. M. Venditti (National Cancer Institute, Bethesda, Md). The spontaneous lymphoma and mammary tumors arose in retired breeder female mice obtained from the Jackson Laboratory, Bar Harbor, Maine.

In Vitro Soft Agar Colony Assay for KHT Tumor Cells

The culture consisted of two layers of agar in a 35-mm Falcon petri dish. For the top and base layers, 0.33% and 0.5% respectively of Bacto-agar (supplied by Difco, Detroit, Mich) in medium CMRL 1066 with 10% heat-inactivated fetal calf serum was used. To 1 ml of preset base layer, 1 ml of 0.33% agar containing appropriate numbers of KHT tumor cells was added. The dishes were kept at room temperature for 20 minutes to allow gelling and then were incubated at 37°C with a constant flow of 10% CO_2 in air. The plates were examined 10 days later under a microscope and colonies with more than 50 cells were counted. Colony plating efficiency of untreated tumor cells was about 15%.

Assay of Agglutination

Single cell suspensions of normal tissues or tumors were prepared by first mincing the tumors into fine pieces with scissors and then pressing the mince through a fine stainless steel screen (200 mesh/inch) with physiologic saline. Cell clumps were removed by passing the cell suspension through a layer of gauze. The cells were washed twice with saline and suspended at a concentration of 2×10^6 cells/ml of saline. The agglutinability of these cells by concanavalin A was tested by adding 0.5 ml of the cell suspension to a 35-mm Falcon plastic petri dish containing 0.5 ml of a 1-mg/ml concanavalin A solution in physiologic saline. The mixture was held at room temperature and the degree of agglutination was estimated 30 minutes later with the aid of a microscope.

Hematologic and Biochemical Studies in the Pig

The hematologic and biochemical studies of the blood of pigs treated with concanavalin A were made with conventional clinical laboratory methods. Normal hematologic values observed (average of five animals) were: white blood cells, 18×10^3 cells/mm^3; red blood cells, 5.8×10^6 cells/mm^3; and platelets, 600,000 platelets/mm^3. Normal blood chemistry values were: urea, 15 mg/100 ml; bilirubin, 0.1 mg/100 ml; alkaline phosphatase, 20 units/100 ml; and serum glutamic oxaloacetic transaminase, 12 units/100 ml.

RESULTS

Comparison of the Sensitivity of Tumor and Hematopoietic CFU to Concanavalin A

The effects of many of the active chemotherapeutic agents on the proliferative capacity of normal hematopoietic and experimental tumor CFU in vivo have been measured with the use of the spleen colony and lung colony assays. Similar procedures were used to measure the effects of concanavalin A on the viability of normal hematopoietic, leukemic, and KHT CFU.

Groups of normal AKR mice were injected with graded doses of concanavalin A intraperitoneally (ip); 24 hours later the animals were killed and the number of CFU in their femurs was determined with the spleen colony assay. The results (fig 1) show that doses up to 10 mg/mouse produced no measurable effect on the content of CFU in the marrow cavity. Similar results were obtained when normal C3H mice were used.

Leukemic AKR mice were prepared by injecting 10^6 leukemic cells iv. Four days later groups of these mice were given graded doses of concanavalin A. They were killed 24 hours later and the content of lymphoma CFU in their femurs was assayed with the spleen colony assay. The results (fig 1) show that a dose of 6 mg/mouse reduced the number of CFU in the marrow by 1 decade.

Mice bearing KHT fibrosarcoma were prepared by injecting 10^5 KHT cells sc. When these cells had grown to tumors of about 8 mm in diameter, 11 days later, groups of the animals were given graded doses of concanavalin A. Cell suspensions of the tumors were prepared

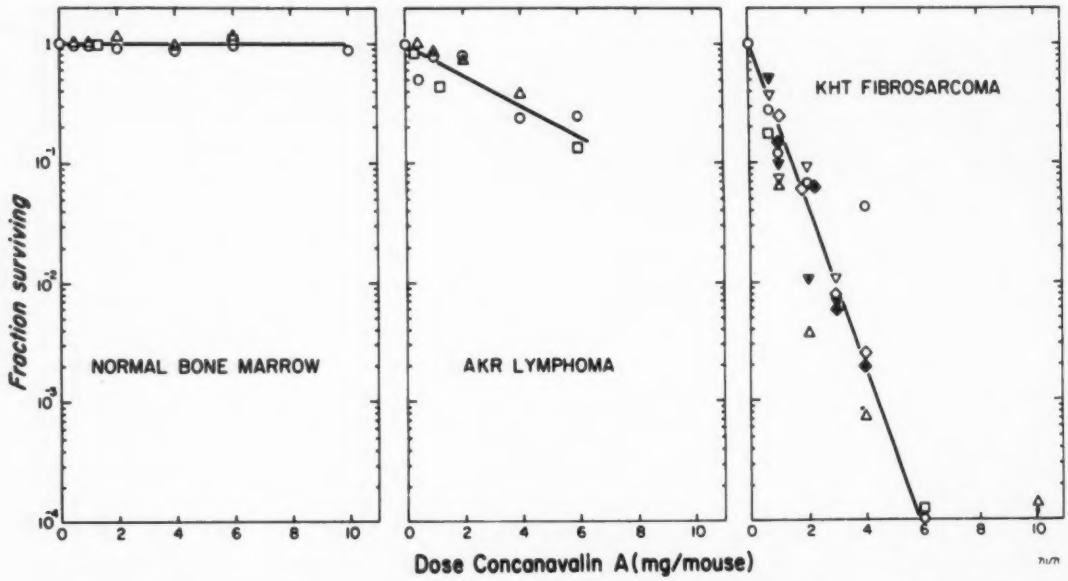

FIGURE 1.—Survival of hematopoietic, AKR lymphoma, and KHT fibrosarcoma CFU 24 hrs after various doses of concanavalin A were administered ip. Each symbol represents an independent experiment. Open symbols represent experiments in which in vivo assay was made. Closed symbols (KHT fibrosarcoma) represent experiments in which the in vitro assay was employed.

and assayed by the lung colony assay 24 hours later. The tumors of animals injected with doses over 1 mg appeared necrotic and hemorrhagic. The assays of the cell suspensions prepared from these tumors showed that they contained many fewer CFU (fig 1). A dose of 1 mg reduced the number of CFU to about 3% and a dose of 6 mg reduced the number to about 0.01% of the number for tumors which were not treated.

The marked selectivity of concanavalin A for KHT, and to a lesser extent AKR lymphoma, compared with its effect on normal marrow is unique (10). However, since concanavalin A, in contrast to previously studied agents, presumably acts on the cell surface, it was possible that the assays did not reflect a true destruction of tumor cells. For instance, it was possible that concanavalin A interfered with the preparation of the cell suspensions, the seeding of the concanavalin A-treated cells to the lung, or the growth of the tumor cells in the new location. To exclude these possibilities, further control experiments were carried out.

Further Studies With KHT Cells

We first examined the effects of concanavalin A given in vivo on the ability of tumor cells to grow in vitro. For this study, KHT-bearing mice were prepared; groups of the animals were given graded doses of concanavalin A and cell suspensions were prepared as described above. The suspensions were then seeded in vitro in soft agar, and after an incubation period of 12 days the survival of cells capable of giving rise to colonies in vitro was measured (closed symbols, fig 1). Clearly, the survival of cells in the tumor suspension was identical for the two (in vivo and in vitro) assay methods.

We next examined the effect of concanavalin A on the survival of KHT cells growing in vitro. A primary culture of sarcoma cells, which had never been grown in tissue culture before, was prepared by mincing the tumor with scissors, forcing the mince through a fine stainless steel screen, and plating the cells on 60-mm Falcon plastic tissue culture dishes with medium containing 10% fetal calf serum. When the cells had nearly formed a confluent mono-

layer, the medium was removed and replaced with medium containing concanavalin A (5–500 μg/ml) in a 2-ml volume. The cultures were incubated for 8 hours after which 2 ml of α-methyl-glucopyranoside (Sigma Chemical Co., St. Louis, Mo) at a concentration of 0.2 M (in medium CMRL 1066 containing 10% fetal calf serum) was added to stop the action of concanavalin A. They were then incubated another 16 hours, the medium was discarded, and the cultures were washed twice on the dishes with Mg^{++} and Ca^{++} free phosphate buffered saline. The cells were removed from the dishes with EDTA solution and were assayed for their colony-forming ability in the lung and in soft agar. The survival of cells per dish capable of forming colonies is shown in figure 2. The results for the two assays were similar. The survival rates at 25 and 50 μg/ml were 25% and 10% respectively. This result showed that concanavalin A could kill KHT cells in vitro under more highly defined conditions than in a tumor.

A third test of the toxic effect of concanavalin A on tumor cells was possible by using a variation of the lung colony assay method in which the tumor cells were exposed to concanavalin A after transplantation. Three groups of mice were each injected with 10^4 KHT cells together with 1×10^6 heavily irradiated (10,000 rads of γ-radiation) tumor cells. The three groups were given 0, 0.5, and 1.0 mg of concanavalin A per mouse ip 24 hours later. Seventeen days later the mice were killed, the colonies in the lungs of each animal were counted, and the survival fraction was determined. The results (table 1) show that concanavalin A reduces the number of colonies that grow from KHT cells exposed to concanavalin A as single cells in the lung. The fraction of CFU surviving agrees well with the survival fraction of cells exposed while the cells were part of a tumor (fig 1).

Taken together, these data demonstrate that concanavalin A can kill KHT cells in vitro and in vivo and that at doses where there is extensive killing of the tumor cells, there is little effect on normal stem cells.

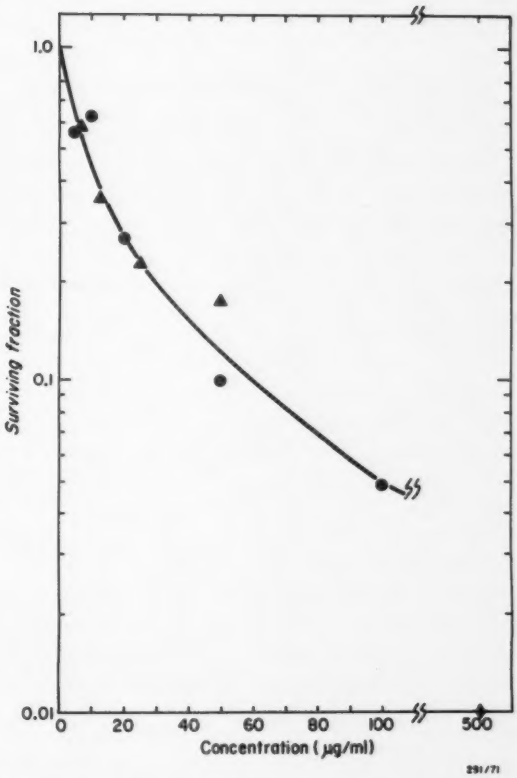

FIGURE 2.—Survival of KHT fibrosarcoma cells which were growing in vitro after 8 hrs' exposure to concanavalin A. Each symbol represents an independent experiment. Closed symbols represent experiments in which assays were performed in vitro. Open symbols represent experiments in which the viability of the cells was assessed by their ability to form colonies in vivo.

Agglutination of Normal and Tumor Cells

We examined the effect of concanavalin A on agglutination to determine whether it showed the same specificity with agglutination as it did with cell killing. Cells from the tissues of normal mice (AKR and C3H) as well as cells from different tumor lines of mice were tested for agglutination in the presence of 500 μg/ml of concanavalin A. Cells prepared with saline only served as controls and the degree of agglutination was scored by two observers using coded preparations. The results (table 2) show that, with the exception of sperm, cells from normal tissues were not agglutinated

TABLE 1.—Effect of concanavalin A on KHT sarcoma cells growing in the lung*

Dose (mg/mouse)	Surviving fractions		
	Experiment 1	Experiment 2	Experiment 3
0	1.0	1.0	1.0
0.5	0.46	0.63	0.58
1.0	0.11	0.18	0.22

*KHT sarcoma cells were injected iv with heavily irradiated cells and 24 hrs later concanavalin A was given ip. After 17 days the No. of colonies in the lungs was counted.

TABLE 2.—Agglutination test for cells of mouse origin

	Cells	Degree of agglutination*
Normal tissues (AKR and C3H)	Thymus, brain, spleen, lung, heart, bone marrow, kidney, lymph node, and testis (other than spermatozoa)	0
	Liver	+
	Spermatozoa	++++
Tumors	Transplanted AKR lymphoma	++
	Spontaneous AKR lymphoma	++
	Transplanted plasma cell tumor	++
	Transplanted C3H mammary tumor	+++
	Spontaneous C3H mammary tumor	+++
	Transplanted KHT sarcoma	++++
	Transplanted Lewis lung tumor	++++

*The degree of agglutination was estimated with a serologic scale of 0 to ++++.

by the protein. In contrast, all tumor cells tested so far have exhibited agglutination. Cells from solid tumors appeared to be more strongly agglutinated by concanavalin A than were the leukemic cells.

These experiments were extended to tissues of human origin using the identical procedures. The results (table 3) show that concanavalin A agglutinates cells of the solid human tumors as strongly as it does the tumors of mouse origin.

Toxicity of Concanavalin A in Mice, Pigs, and Monkeys

The efficacy of a chemotherapeutic agent depends, of course, on its effect on both the tumor and the host, and the toxic effects are usually not limited to the hematopoietic tissue. Since concanavalin A is known to agglutinate red cells of certain species, we re-examined this hemagglutination before we chose a species for toxicity studies since we anticipated that toxicity would be associated with hemagglutination.

Blood from several species was obtained in an anticoagulant; it was washed and assayed for its agglutination by concanavalin A. The results (table 4) were expressed as the lowest concentration of concanavalin A required to give complete hemagglutination. They confirm the marked range of hemagglutination with concanavalin A observed previously (5). For instance, agglutination of human and pig blood requires 1000 times the concentration that it does for monkey or mouse blood. We might thus expect that concanavalin A would be much

TABLE 3.—Agglutination test for cells of human origin

Tumor cells	No. of cases	Degree of agglutination*
Chronic myelogenous leukemia	1	++
Acute myeloblastic leukemia	2	++
Multiple myeloma	1	++
Hodgkin's disease	1	++
Astrocytoma	1	++
Prostatic carcinoma	1	++
Mammary carcinoma	2	+++
Bronchogenic carcinoma	2	++++

*The degree of agglutination was estimated with a serologic scale of 0 to ++++.

TABLE 4.—Hemagglutination test

Source of blood	Minimum concentration of concanavalin A (μg/ml)
Man	>5000
Sheep	>5000
Pig	>5000
Mouse (AKR, C3H)	5
Monkey	5
Baboon	5
Horse	0.5
Chimpanzee	0.5

less toxic in men and pigs than it would be in monkeys and mice.

The toxicity of concanavalin A given as a single dose to mice, pigs, and monkeys was therefore determined. In brief, the approximate LD50s for these species were as follows: mouse, 150 mg/kg ip and 15 mg/kg iv (based on 20 animals); pig, 5 mg/kg iv (based on 13 animals); and monkey, 5 mg/kg iv (based on three animals). Clearly, the toxicity to the three species was similar and there was no correlation of toxicity with hemagglutination. More detailed study did not reveal that hemagglutination has an important role in the toxicity.

Mice died after a prolonged period of from 12 to 48 hours after the administration of concanavalin A. Mice bearing KHT tumors appeared somewhat more sensitive than normal animals.

Pigs were given doses of 1, 3, or 10 mg/kg of concanavalin A iv in 45 seconds. At all doses the animals began vomiting for a short period about 15 minutes after the injection, and at doses of 3 and 10 mg/kg they had diarrhea which lasted several hours. Animals receiving 10 mg/kg became dyspneic after 4 hours and died within 24 hours. The most striking feature upon necropsy of animals injected with 3 mg/kg 24 hours earlier was a marked subepithelial hemorrhage in the duodenum and jejunum extending from a sharp border at the pylorus. At the higher doses there was pulmonary edema and evidence of right heart failure. Microscopic examination showed a vasculitis evident in both the small intestine and the lung as well as in the kidney, liver, and heart. The marked pathologic changes at 24 hours were not seen at later times nor were they reflected in blood chemistry tests or hematologic parameters (*see* Materials and Methods) at 1 week to 1 month after a 3-mg/kg dose.

Monkeys were injected with doses of 1, 3, and 10 mg/kg. There was neither immediate vomiting nor diarrhea although the animals receiving 10 mg/kg died with a hemorrhagic lesion in the small intestine similar to that observed in the pig.

DISCUSSION

The selectivity of concanavalin A is clearly evident from these studies. Concanavalin A selectively kills mouse tumor cells as compared with normal hematopoietic cells. This selectivity is most impressive when compared with the selectivity displayed by most chemotherapeutic agents (10). None of the chemotherapeutic agents available at present show a selective killing of solid tumors without affecting the hematopoietic stem cells when the chemotherapeutic agents are given systemically. The unique mechanism for the selectivity is not known. It would appear reasonable that it is closely related to the selectivity in the binding of concanavalin A associated with agglutination, since there is a good correlation between

the degree of cell agglutination and the in vivo and in vitro cell killing by concanavalin A.

If there is a general correlation between agglutination and killing of cells, then human tumor cells which are agglutinated by concanavalin A in vitro should be killed by concanavalin A in vivo. But it would appear that the use of concanavalin A as a cancer chemotherapeutic agent would be severely limited by its peculiar toxicity (14). It remains to be seen if it will be possible to alter its structure or to obtain other lectins which possess the selective properties of concanavalin A but do not produce the generalized vasculitis.

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Imidazole Carboxamides: Relationship of Lipophilicity to Activity Against Intracerebral Murine Glioma 26 and Preliminary Phase II Clinical Trial of 5-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) in Primary and Secondary Brain Tumors [1,2,3,4]

Victor A. Levin,[5] David Crafts,[5] Charles B. Wilson,[5] Pokar Kabra,[5] Corwin Hansch,[6] Edwin Boldrey,[5] Jean Enot,[5] and Marguerite Neely [5,7]

To date, the most active chemotherapeutic drugs against brain tumors have been lipophilic and cell-cycle nonspecific (CCNS) in their mode of action (1). The 5-[3,3-disubstituted-1-triazeno]imidazole-4-carboxamides belong to a family of compounds whose chemotherapeutic action is probably CCNS (2,3) although the active constituent is unknown. These compounds are slow in reaction and require extensive metabolism to yield potentially active moieties (2,3). Monofunctional alkylation may be the active product of imidazole carboxamide metabolism but this awaits verification (3). Because many of the imidazole carboxamides are lipophilic and might be potential chemotherapeutic agents for brain tumors, these studies were designed to evaluate log P and antitumor activity in intracerebral (ic) Glioma 26. The objective was to identify compounds active against brain tumors. In addition, a preliminary phase II study was designed to evaluate the activity of 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) in brain tumors since this is the only lipophilic imidazole carboxamide available for clinical testing.

MATERIALS AND METHODS

C57BL/6 mice bearing ic Glioma 26 tumors were treated on Day 12 after tumor implantation with one of seven imidazole carboxamide compounds (table 1) suspended in sesame oil. Each animal received 0.1 cc/10 g of body weight.

Patients selected for treatment with NSC-82196 fulfilled the following criteria: (*a*) had primary or secondary tumor of the brain confirmed by prior surgical biopsy or subsequent autopsy, (*b*) had progressive neurologic deterioration while receiving nonescalating doses of dexamethasone,[8] (*c*) at least 3 months had elapsed since radiotherapy, (*d*) at least 2 months had elapsed since surgery, and (*e*) had had no previous chemotherapy for 8 weeks prior to NSC-82196 administration.

The initial evaluation consisted of: (*a*) a general physical and neurologic examination; (*b*) complete blood cell counts, SMA-12, urinalysis, technetium 99m brain scintiscan, and electroencephalogram; and (*c*) contrast radiog-

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[2] Supported by Public Health Service grant CA-13525 (Brain Tumor Research Center), grant CA-15435, and contract N01-CM-33703 (Division of Cancer Treatment [DCT]) from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] NSC-82196: CAS reg. No. 5034-77-5; BIC. Supplied for animal studies by Dr. Robert Engle of Drug Research and Development (DRD), DCT, NCI, and for clinical studies by Cancer Therapy Evaluation (CTE), DCT, NCI.

[4] Six of the triazenes were synthesized by Charles L. Schmidt and Stanley Milstein under contract N01-CM-33703 (Dr. R. Nelson Smith, Principal Investigator).

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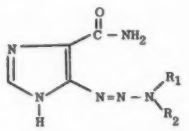
[8] Dexamethasone: NSC-34521; CAS reg. No. 50-02-2; pregna-1,4-diene-3,20-dione, 9-fluoro-11β,17,21-trihydroxy-16α-methyl-.

TABLE 1.—Imidazole carboxamide chemotherapy of ic Glioma 26 on Day 12 after tumor implantation—relationship of dose to log P



R_1=	R_2=	Log P	Dose range (mmol/kg)	Maximum T/C (%)
Methyl	Methyl	−0.24	0.41–2.06	120
Methyl	Propyl	0.79	0.24–1.43	112
Methyl	Butyl	1.26	0.22–1.34	108
Methyl	Benzyl	1.54	0.20–1.22	126
Chloroethyl	Chloroethyl	1.54	0.48–1.43	135
Methyl	Phenethyl	2.04	0.36–1.65	144
Methyl	Octyl	3.25	0.18–1.61	166

raphy tests (arteriography, pneumoencephalography, and ventriculography) when indicated.

NSC-82196 was prepared fresh (1 g/48.5 cc, 0.01N HCl diluent) and administered by rapid intravenous injection over 30 minutes at a dose of 900 mg/m²/day for 5 consecutive days. Treatment was repeated every 6 weeks. The initial examination and tests (exclusive of radiography) were repeated every 6 weeks. At the time of re-treatment, dose adjustment was made for myelotoxicity.

Response to NSC-82196 was defined as an unequivocal clinical neurologic improvement *and* an unequivocal isotope scintiscan improvement during the same or lower maintenance doses of dexamethasone. Probable responders were defined as those patients who demonstrated no deterioration in clinical neurologic scans and isotope brain scintiscans for 3 months or longer during nonescalating doses of dexamethasone.

RESULTS

Table 1 summarizes the results obtained in ic Glioma 26 with the seven imidazole carboxamides tested. For each drug there were four to seven different doses given; each experiment consisted of nine to ten animals at each dose level. Although Hansch's analysis of log P versus antitumor activity was not possible (4), the data indicate that increasing lipophilicity is associated with better survival.

Studies with NSC-82196 in man are summarized in table 2. A total of 24 patients were consecutively accrued. They represented 16 patients who had evidence of recurrent tumor but who had not received previous chemotherapy, six patients who had received a single previous form of chemotherapy, one patient who had received two previous forms of chemotherapy, and one patient who had received three previous forms of chemotherapy. Ten patients either died or were in a terminal status and did not receive a second course of NSC-82196. Of the 16 patients who received NSC-82196 as their first form of chemotherapy, four (25%) had a definite response with a median duration of response of more than 5 months. If those patients who failed to receive two courses of NSC-82196 therapy were eliminated, the response rate was 44% (four of nine patients). Assuming that the astrocytoma patients were actually harboring malignant gliomas at the time of chemotherapy, the response rate for all malignant gliomas was 21% (four of 19 patients); when patients who received fewer than two courses of NSC-82196 were eliminated the response rate was 31% (four of 13 patients). Interestingly, two of six (33%)

TABLE 2.—Clinical response to NSC-82196 by tumor type and previous exposure to chemotherapy

Response to NSC-82196	Disease*								Total
	Glioblastoma multiforme	Malignant glioma	Astrocytoma†	Oligodendroglioma	Medulloblastoma	Reticulum-cell sarcoma	Ependymoma (brain stem)	Adenocarcinoma (lung)	
Definite response: previous treatment									**6**
None	—	2 (5,5.5)	—	1 (7)	—	—	—	1 (5)	
1 course	1 (11.5)	—	—	—	—	1 (17+)	—	—	
2 courses	—	—	—	—	—	—	—	—	
3 courses	—	—	—	—	—	—	—	—	
Probable response: previous treatment									**1**
None	—	—	1 (3)	—	—	—	—	—	
1 course	—	—	—	—	—	—	—	—	
2 courses	—	—	—	—	—	—	—	—	
3 courses	—	—	—	—	—	—	—	—	
No response (<2 courses): previous treatment									**11**
None	1	4	1	—	—	—	1	—	
1 course	1	2	—	—	—	—	—	—	
2 courses	—	—	—	—	—	—	—	—	
3 courses	—	1	—	—	—	—	—	—	
No response (>2 courses): previous treatment									**6**
None	3	—	1	—	—	—	—	—	
1 course	—	1	—	—	—	—	—	—	
2 courses	—	—	—	—	1	—	—	—	
3 courses	—	—	—	—	—	—	—	—	

*Nos. in parentheses = duration of response (mos).

†These tumors were diagnosed as low-grade astrocytomas >9 mos before NSC-82196 therapy; based on the rapid down-hill course of these patients it is probable that these tumors were malignant, from a cell kinetic point of view, at the time of NSC-82196 treatment.

patients receiving NSC-82196 as a second form of chemotherapy responded for 11.5 and 17+ months.

DISCUSSION

The antitumor activity of seven imidazole carboxamides, varying in log P from –0.24 to 3.25, was evaluated using ic Glioma 26. It was found that activity increased with increasing lipophilicity and that the most active (treated vs control [T/C] = 135%–166%) imidazole carboxamides appeared between log P 1.54 and 3.25. Because of the limited activity of these agents, a parabolic or linear fit of the data could not be made.

Hansch et al compared log P to antitumor activity of 21 carboxamides in L1210 leukemia and found 11 active drugs (4). From these structure-activity studies they concluded that one of the substitutions of the triazene should contain a methyl group for activity (the exception was NSC-82196). They also found that optimum activity occurred at log P ~ 1. In comparison, they noted that the optimum activity of the nitrosoureas in L1210 leukemia was at log P ~ –0.6. Subsequent to this, Montgomery et al compared the activity of 17 nitrosoureas in the Lewis lung tumor and found optimum activity at log P ~ 0.8 (5). We compared the activity of six nitrosoureas against ic sarcoma 9L and found optimum activity at log P ~ 0.4 (6). From these studies and the results of our present experiments in Glioma 26, it is tentatively concluded that (*a*) solid tumors require more lipophilic drugs than leukemias, (*b*) effective imidazole carboxamides must be more lipophilic than the nitrosoureas to exert maximal antitumor activity, and (*c*) the nitrosoureas are at least twice as active as the imidazole carboxamides.

Both the dimethyl compound (5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide [NSC-45388])* and the bis(2-chloroethyl) compound (NSC-82196) are clinically available. Both have shown activity in melanoma, although NSC-45388 is judged somewhat superior to NSC-82196. The use of NSC-45388 in brain tumors has consisted of three pilot studies. The first study was that of Kingra et al who evaluated five patients with low-grade tumors, three of whom had partial responses (7). The second study was that of Taylor et al, who used NSC-45388, NSC-45388 plus CCNU, or NSC-45388 plus methyl-CCNU in combination with surgery and radiation (8).[10] They had eight patients who appeared to fit most of our criteria and could be compared to the present study. Of these eight patients treated with NSC-45388 alone, five responded with a median response interval of 4 months. We have used NSC-45388 in more than 15 patients in phase II studies without observing significant activity. However, most of these patients had been treated earlier with another agent. We observed one response in eight evaluable patients who had received prior chemotherapy with another agent.[11]

We evaluated NSC-82196 (rather than NSC-45388) because of the experimental animal data which indicated greater activity of more lipophilic drugs. Unfortunately, none of the other imidazoles in table 1 is available for clinical testing. Because NSC-82196 is 20%–25% ionized at physiologic pH and has demonstrated limited blood-cerebrospinal fluid exchange (9), the methyl-octyl substituted triazene would have been a better choice. Nonetheless, the data summarized in table 2 indicate that NSC-82196 is clinically active against brain tumors. If those patients manifesting rapid tumor regrowth are eliminated (ie, those dead or in terminal status before the second course), the definite response rate for all patients was 46% (six of 13) and one of these 13 patients had a probable response of 3 months. In the glioma series (glioblastoma multiforme, malignant

*NSC-45388: CAS reg. No. 4342-03-4; DTIC.

**CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-; Me-CCNU.

glioma, astrocytoma, and oligodendroglioma) four of ten (40%) patients receiving more than two courses of NSC-82196 responded (plus one probable responder) with a median duration of response of more than 5 months. Although NSC-82196 demonstrates activity against brain tumors in man, it does not seem to be as active as BCNU [11,12] (1) and, clearly, is more difficult to administer.

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[11] Wilson CB, Gutin P, Kumar ARV, et al. Single agent chemotherapy of brain tumors: a 5-year review. Submitted to Arch Neurol for publication.

[12] BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

Phase II Trials With Procarbazine (NSC-77213), Streptozotocin (NSC-85998), 6-Thioguanine (NSC-752), and CCNU (NSC-79037) in Patients With Metastatic Cancer of the Large Bowel [1,2]

J. Horton, A. Mittelman, S. G. Taylor III, L. Jurkowitz, J. M. Bennett, E. Ezdinli, J. Colsky, and J. A. Hanley [3,4]

SUMMARY

One hundred and ninety-seven patients with measurable metastatic cancer of the colon have been treated with one of four anticancer drugs which have had little prior trial in this disease. Objective tumor responses lasting a median of 9 weeks occurred with 0.5 g/m² of streptozotocin given intravenously every week (10%), 130 mg/m² of CCNU given orally every 6 weeks (10%), 1.0 mg/kg/day of 6-thioguanine given orally (8%), and 3 mg/kg/day of procarbazine given orally (3%). Performance status declined more rapidly with streptozotocin and 6-thioguanine and the median survival time was less (12 and 16 weeks respectively) than with procarbazine and CCNU (23 and 20 weeks respectively). This study suggests that procarbazine given in this way is ineffective but trials of streptozotocin or 6-thioguanine combined with other agents active against colon cancer should ensue as well as further exploration of the usefulness of other nitrosoureas.

[Cancer Chemother Rep 59:333–340, 1975]

[1] Received May 3, 1974; revised Sept 25, 1974; accepted Nov 20, 1974.

[2] Compounds used in this study were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute (NCI). Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

[3] This study was done by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman) and was supported by Public Health Service grants from the NCI, National Institutes of Health, Department of Health, Education, and Welfare. The following institutions participated in the study: Albany Medical College of Union University, NY (*J. Horton*; CA-06594); Roswell Park Memorial Institute, Buffalo, NY (*A. Mittelman*; CA-12296); Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill (*S. G. Taylor III*; CA-10948); Maimonides Medical Center, Brooklyn, NY (*L. Jurkowitz*; CA-05588); University of Rochester, NY (*J. M. Bennett*; CA-11083); Chicago Medical Center at Mt. Sinai Hospital Medical Center, Ill (*E. Ezdinli*; CA-14144); Jackson Memorial Hospital, Miami, Fla (*J. Colsky;* CA-02822); State University of New York at Buffalo, Amherst (*J. A. Hanley*). Other participating institutions were: Albert Einstein College of Yeshiva University, University of Alberta (CA-13314), American Oncologic Hospital, Brookdale Hospital Center, Boston University Medical Center (CA-11109), Case Western Reserve, Georgetown University School of Medicine (CA-02824), Hahnemann Medical College (CA-13611), Jefferson Medical College (CA-14215), Lahey Clinic Foundation (CA-12880), University of Manitoba, Mt. Sinai (New York), University of Ottawa, Universitaire Lariboisier (Paris), Pennsylvania Hospital (CA-13613), University of Pennsylvania, Tufts University School of Medicine (CA-07190), and Medical College of Virginia (CA-10572).

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Cancer of the colon and rectum is relatively resistant to chemotherapeutic attack by currently available agents. Presently, 5-fluorouracil is the most commonly used and probably most effective agent available but, in our hands, only approximately 20% of patients treated have had objective tumor shrinkage (1,2). In addition, studies of its use in the early stages of disease as an adjuvant to surgery have not conclusively demonstrated its effectiveness (3). In contrast to reports of increased effectiveness of drug combinations in Hodgkin's disease, leukemia, and breast cancer (4), our experience with several combinations in colon cancer (5–8) has been poor and more effective agents are needed. We decided to test several drugs for activity in patients with colorectal cancer that were known to be active in other tumor types but which had not previously received an adequate trial in this disease. Thus, CCNU was chosen for study because of its activity in lymphoma and because of some encouraging results in large bowel cancer from another nitrosourea, BCNU (9); streptozotocin was chosen because of its lack of marrow toxicity and effectiveness in islet-cell tumors and carcinoid syndrome (10); and procarbazine and 6-thioguanine were chosen because of their proven effectiveness in lymphoma (11) and leukemia (12) respectively.

METHODS

Patients were entered into this study by members of the Eastern Cooperative Oncology Group (ECOG) if they had histologically confirmed metastatic cancer of the colon or rectum that was no longer amenable to standard surgical or radiotherapeutic treatment techniques. Patients were considered eligible for the study if they had components of their disease that could be measured (provided that they were expected to survive at least 90 days), and had a starting white blood cell (wbc) count of at least 5000 cells/mm^3, a platelet count of at least 150,000 platelets/mm^3, a blood urea nitrogen level less than 25 mg/100 ml or a creatinine level less than 1.5 mg/100 ml, and a bilirubin level less than 2.0 mg/100 ml. Those patients who had not recovered from any effects of major surgery or had significant infection were specifically excluded from the study. Patients were also ineligible if they had been previously treated with any of the drugs being studied or had received extensive pelvic irradiation. All patients had given their written informed consent for this study.

The initial patient evaluation included clinical examination and measurement of tumors as well as a complete blood and platelet count, blood urea nitrogen or serum creatinine, serum bilirubin, alkaline phosphatase, urinalysis, and chest roentgenogram. The blood count was repeated at weekly intervals in most patients and the baseline studies and tumor measurements were repeated at least monthly as well as at the end of the study. Notation of these parameters as well as a recording of performance status were made on special flow sheets.

Tumor size was arbitrarily defined as the sum of the product of two perpendicular diameters of all measured lesions. A complete regression represented the disappearance of all disease and a return to normal of any abnormal laboratory parameter. A partial response was defined as at least a 50% decrease in the sum of the product of tumor diameters in the absence of progression of any lesion. The category of no change represented less than a 50% decrease and progression was defined as an increase greater than 25%. For those patients in whom an enlarged liver due to metastasis was used as the measurable parameter, it was necessary that a clearly defined edge extended at least 5 cm below the costal margin or xiphoid process. A partial response was demonstrated if the sum of the measurements below the xiphoid and both costal margins at the midclavicular lines decreased by at least 30%. Liver scans were not used as a criterion of response. The scale used for the classification of performance status by the ECOG was:

0—fully active;

1—ambulatory, capable of light work activities;

2—in bed less than 50% of the time, capable of self-care but not of work activities;

3—in bed greater than 50% of the time, capable of only limited self-care;

4—completely bedridden.

Toxicity was graded as is shown in table 1.

TABLE 1.—Classification of severity of toxicity

Toxic effects	Degree		
	Mild-moderate	Severe	Life threatening-fatal
Hematologic Wbc count (cells/mm³)	≥1500	1000–1499	<1000 or <1500 associated with infection
Platelet count (platelets/mm³)	≥50,000	30,000–49,999	<30,000 or <50,000 associated with bleeding
Vomiting	Intermittent, controllable by antiemetics	Continuous, only partly controllable by antiemetics	Continuous and uncontrollable
Stomatitis	Mild to moderate ulceration	Severe ulceration of entire mucosa	Superadded necrosis of mucous membranes and deeper tissues
Renal	Reversible mild to moderate impairment of renal function	Marked compromise of renal function	Irreversible marked impairment of renal function

TABLE 2.—Modifications of drug dosage for hematologic and gastrointestinal toxic effects

Dose modification (% of planned dose)	Wbc count (cells/mm³)	Platelet count (platelets/mm³)	Stomatitis	Vomiting
100	>4500	>150,000	None	None or mild
50	3000–4499	75,000–149,999	Minimal	Moderate
0	<3000	<75,000	Severe	Severe

Each patient received treatment which was assigned on a random basis by means of treatment cards which were enclosed in sealed envelopes and drawn by the investigator immediately prior to the patient's entry into the study. Each institution received a separate set of sealed envelopes containing the random treatment assignments to be used for successive patients entered but the specific subset of treatments varied from institution to institution.

The four drugs used in this study were: CCNU (130 mg/m²) given orally as a single dose and repeated every 6 weeks; 6-thioguanine (1.0 mg/kg/day) given orally in two divided doses; procarbazine (100 mg/day) given orally for 7 days and then daily (3 mg/kg); and streptozotocin (2.0 g/m²) given intravenously (iv) weekly. Early in the study the dosage of streptozotocin was reduced to 0.5 g/m² weekly. Treatment was planned to continue for 9 weeks. If no change or clinical benefit occurred, treatment could be continued indefinitely. Modifications for hematologic and gastrointestinal toxic effects were as indicated in table 2. Patients treated with streptozotocin had their dosage interrupted if albuminuria developed or progressed, or if azotemia progressed.

RESULTS

Patients

Of 257 patients entered into this study, 222 have completed the study and have been evaluated. Twenty-five patients are not included in this analysis because of ineligibility (eight patients), inadequate treatment or followup (ten patients), or protocol violation (seven patients). Patients were entered from 25 cooperating institutions but the main patient accrual was from Roswell Park Memorial Institute, University of Rochester, Albany Medical College, Mt. Sinai Hospital Medical

Center (Chicago), Jackson Memorial Hospital, and Rush-Presbyterian-St. Luke's Medical Center.

The distribution of age and sex was similar in each treatment group. Sixty-seven percent of the patients had received prior chemotherapy, mostly with 5-fluorouracil, and this factor was similar in each group. Ninety-four patients (48%) were initially ambulatory. Of the patients who received CCNU, proportionately fewer (38%) were ambulatory at the beginning of therapy compared with 59% who were ambulatory in the group who received 6-thioguanine.

Toxic Effects

The incidence and degree of toxic reactions experienced by patients treated with each drug are summarized in table 3.

Procarbazine was generally well tolerated. Hematologic toxic effects were usually mild and gastrointestinal reactions were rarely severe. Streptozotocin complications were characterized by vomiting which occurred in 35 of the 50 patients treated and were considered severe in nine. Severe or lethal renal toxic effects occurred in four patients. One of the four patients had received 2 g/m² of streptozotocin and the renal toxic reaction was fatal, while severe renal toxic manifestations were encountered at the lower dose level of 0.5 g/m²/week in the other three patients. A further drug-related death at this dose level was attributed to infection and pulmonary failure. Nine of 55 patients treated with CCNU had severe hematologic toxic reactions, mostly thrombocytopenia. In addition, 29% of those treated experienced some gastrointestinal reactions which were usually minor in degree. Hematologic toxic reactions (both leukopenia and thrombocytopenia) from 6-thioguanine were severe in four patients, life threatening in three patients, and gave rise to two drug-related deaths. In four of these nine patients the myelosuppression resulted in serious infection and in another patient it was accompanied by increased bilirubin levels. Gastrointestinal tolerance was similar to that for CCNU.

Objective Tumor Response

The incidence of antitumor responses is listed in table 4. The overall response rate was 8%. Differences between the observed responses to streptozotocin, CCNU, and 6-thioguanine are not significant but these three treatments do appear to be somewhat more active than pro-

TABLE 3.—Summary of incidence and severity of toxicity induced by each drug treatment

Drug	No. of patients	Toxic effects	Severity of toxic effects—No. of patients with—			
			None	Mild-moderate	Severe	Life threatening-fatal
Procarbazine	38	Hematologic	23	12	3	0
		Vomiting	26	9	3	0
		Stomatitis	35	2	1	0
		Renal	38	0	0	0
Streptozotocin	50	Hematologic	50	0	0	0
		Vomiting	15	26	9	0
		Stomatitis	49	1	0	0
		Renal	44	2	2	2
CCNU	55	Hematologic	32	13	4	5
		Vomiting	38	15	1	0
		Stomatitis	50	3	1	0
		Renal	55	0	0	0
6-Thioguanine	54	Hematologic	36	9	4	5
		Vomiting	38	14	2	0
		Stomatitis	51	3	0	0
		Renal	54	0	0	0

carbazine. The durations of remission ranged from 2 to 60 weeks with a median duration of 9 weeks. The small number of remissions that we encountered do not allow intertreatment comparisons of the length of remissions.

Table 5 demonstrates the relationship of prior chemotherapy to the expectation of response to the drugs studied.

The responses to CCNU occurred more often in previously untreated patients but those responses to streptozotocin occurred in patients who had received prior chemotherapy. When the patients who were initially ambulatory (ECOG performance status 0 or 1) are considered separately from those who were not, the former group had a significantly greater frequency of tumor response ($P = 0.03$). This is shown in table 6.

Symptomatic Response

Notation was made of the changes in performance status during treatment. Table 7 indicates the change in performance status that occurred after 10 weeks of treatment. A greater number of patients who were initially ambulatory and who received procarbazine or CCNU remained in an ambulatory status ($P = 0.1$). Patient improvement from a nonambulatory status occurred most frequently with 6-thioguanine and then, to a lesser extent, with CCNU.

Survival

Overall survival rates are indicated in table 8. The differences in patient survival among the four treatment groups were associated with a significance level of $P = 0.08$, suggesting that the observed variations were not due to chance alone. The survival times for patients who had received prior chemotherapy were similar to those for patients who had not (median, 17.7 versus 16.6 weeks respectively). Streptozotocin was associated with the shortest survival rate in both instances.

The patients who were initially ambulatory had a median survival time of 21.5 weeks whereas those who were initially nonambulatory had a median survival time of 12.9 weeks. Similarly, the median survival time for all responders was 25.0 weeks in comparison with 16.5 weeks for nonresponders and 17.4 weeks for the entire group.

DISCUSSION

The design of this study allowed simultaneous comparison of the four drug treatments. The distribution of patient characteristics, particularly with respect to initial performance

TABLE 4.—Incidence of objective tumor response

Treatment	Response—No. of patients with—				
	Complete	Partial	No change	Progression	Totals
Procarbazine	0	1	17	20	38
Streptozotocin	0	5	11	34	50
CCNU	0	6	8	41	55
6-Thioguanine	2	2	15	35	54
Totals (%)	2(1)	14(7)	51(26)	130(66)	197(100)

TABLE 5.—Tumor response in relation to prior chemotherapy

Treatment	Previously untreated		Previously treated	
	No. of responses	Total No. of patients	No. of responses	Total No. of patients
Procarbazine	0	13	1	25
Streptozotocin	0	17	5	33
CCNU	5	18	1	37
6-Thioguanine	1	17	3	37
Totals	6(9%)	65	10(8%)	132

TABLE 6.—Objective tumor response in patients initially ambulatory or nonambulatory*

Treatment	Ambulatory		Nonambulatory	
	No. of responses	Total No. of patients	No. of responses	Total No. of patients
Procarbazine	1	18	0	16
Streptozotocin	3	23	2	23
CCNU	5	21	0	32
6-Thioguanine	2	32	1	19
Totals	11(12%)	94	3(3%)	90

*Includes only patients on whom all appropriate data were available.

TABLE 7.—Change of performance status from the start of therapy to 10 weeks after the start of therapy*

Treatment	Initially ambulatory—performance status 0 or 1		Initially nonambulatory—performance status 2 or 3	
	No. of patients	No. of patients still ambulatory at 10 wks	No. of patients	No. of patients who improved to ambulatory at 10 wks
Procarbazine	14	10	13	1
Streptozotocin	20	9	22	0
CCNU	19	13	27	5
6-Thioguanine	29	11	19	9
Totals	82	43	81	15

*Includes only patients on whom all appropriate data were available.

status, was not homogenous. This occurred because each institution involved in the study had, initially, a choice of testing two, three, or all four of the drug treatments although once they had made this choice the randomization in this subset of treatments was binding.

The data demonstrate that ambulatory patients were more likely to obtain objective tumor responses than nonambulatory patients (12% versus 3%) and that these initially ambulatory patients had a longer expectation of survival (median, 21.5 versus 12.9 weeks). Thus, on the basis of this factor alone, patients who received 6-thioguanine should have done better in terms of tumor response and survival since there were a greater number of patients who were initially ambulatory in this group. The rate of decline for patients with initially good performance status was slowest for procarbazine and CCNU. This probably indicates that these two drugs were tolerated better than streptozotocin and 6-thioguanine. Only 12% of the patients who were initially nonambulatory improved to an ambulatory status after 10 weeks, and this improvement was noted most often with 6-thioguanine (50%) and CCNU (20%).

There was no significant difference between the incidence of antitumor responses from streptozotocin, CCNU, and 6-thioguanine although each was significantly more active than procarbazine. A concomitant "control" arm of 5-fluorouracil alone was not made part of this study. The relationship of expectation of response with prior chemotherapy was different for all the active drugs. Most responses from CCNU occurred in patients who did not have prior treatment whereas streptozotocin activity was greatest in previously treated patients and 6-thioguanine had some activity in both groups. It is generally accepted that the expectation of objective response to a chemotherapeutic

TABLE 8.—Median survival of patients treated measured from the onset of treatment

Treatment	No. of patients treated	No. of deaths	Median survival (wks)
Procarbazine	38	27	22.6
Streptozotocin	50	41	12.1
CCNU	55	42	20.2
6-Thioguanine	54	42	16.2
Totals	197	152	

agent in many tumor types is less if this chemotherapy is given after other chemotherapy has been tried. It is perhaps unusual that in this study the overall incidence of remissions was similar for both previously treated and untreated patients. Only two patients had complete remissions; both were treated with 6-thioguanine.

The incidence of objective responses noted for streptozotocin in this study is not significantly different from that noted by Moertel et al (12) although Stolinsky et al (13) had no responses in three patients treated. Similarly, the results obtained with CCNU agree closely with those of Moertel et al (14) but Hoogstraten et al (15) reported no objective responses in 16 patients treated.

The degree of hematologic toxic effects induced by the dosage of 6-thioguanine used was unexpectedly high since approximately double this dosage is customarily used in antileukemic regimens (12). Since this was the only agent that induced any complete remissions, further study of its combination with 5-fluorouracil is indicated. Similarly, the lack of hematologic toxic effects from streptozotocin suggests that combination of this agent with 5-fluorouracil might give additive antitumor effect without prohibitive toxicity. The demonstration of activity of CCNU suggests that other related compounds such as methyl-CCNU, which has increased activity in some animal tumor systems (13), should be tested in human colon cancer. These studies are presently under way.

LIST OF COMPOUNDS

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-*α*-(2-methylhydrazino)-, monohydrochloride

Streptozotocin: NSC-85998

6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-; 6TG

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Clinical Trials With 1-Acetyl-2-picolinoylhydrazine (NSC-68626) in Children[1,2,3,4]

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SUMMARY

Thirty-seven children with various malignant neoplasms were treated with 1-acetyl-2-picolinoylhydrazine. Complete regressions were documented in one child with embryonal rhabdomyosarcoma, in one child with generalized histiocytosis-X, and in one child with Hodgkin's disease. One patient had renal failure while receiving the drug but no conclusive cause and effect relationship could be established. Toxic effects otherwise were mild and consisted primarily of nausea and vomiting.

[Cancer Chemother Rep 59:341–344, 1975]

1-Acetyl-2-picolinoylhydrazine, a synthetic compound, was investigated in clinical phase II trials in children who had advanced malignant disease because of its availability in an oral form for administration, lack of bone marrow suppressive toxic manifestations, and reported antineoplastic activity in patients with rhabdomyosarcoma and breast cancer (1). This synthetic compound produced an 80%–100% inhibition of Walker 256 tumor cells transplanted into rats (2).[10] In mice and rats, the drug was equally active when administered orally, subcutaneously, or intraperitoneally.[10]

MATERIALS AND METHODS

Children under 15 years of age who had malignant tumors of various histologic types were registered for the study if they had progressive disease after prior treatment with conventional drug regimens. Prestudy evaluation included assessment of renal, bone marrow, and hepatic status, as well as documentation of the extent of the disease. Regular followup examinations were conducted at least once a week to document antitumor effect and toxicity.

1-Acetyl-2-picolinoylhydrazine was adminis-

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[3] 1-Acetyl-2-picolinoylhydrazine was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

[4] This study was performed by the Southwest Oncology Group (SWOG Protocol 368).

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[10] Livingston RB, Carter SK, Davignon JP, et al. Clinical brochure: 1-acetyl-2-picolinoylhydrazine, NSC-68626. Prepared by the NCI, Bethesda, Md, Aug 1968, 17 pp.

tered at a dose of 325 mg/m²/day orally either as a single dose or in two divided doses. A full course consisted of 28 doses. If a response occurred, the drug was continued in the same dose for the duration of the response.

RESULTS

The results of this study are summarized in table 1. A total of 37 patients were registered in the study. Six patients died of progressive disease after receiving from two to 12 daily doses of the drug; these patients were arbitrarily categorized as "early deaths." Because of rapidly progressive tumor spread, three other patients were removed from the study after 21, 24, and 25 days respectively. All of the remaining patients received courses of therapy that consisted of 28 or more days of 1-acetyl-2-picolinoylhydrazine administration.

Table 1 shows a breakdown by histologic tumor types, the number of patients in each category, and the number of responses. A response was considered to be complete when there was disappearance of all objective evidence of tumor.

Complete regressions occurred in one child with embryonal rhabdomyosarcoma (duration, 198 days), in one child with generalized histiocytosis-X (duration, 162 days), and in one child with Hodgkin's disease (duration, 312 days). The clinical histories on these three patients who had objective antitumor responses after therapy with 1-acetyl-2-picolinoylhydrazine are summarized below.

Patient No. 1

A 5-year-old boy with embryonal rhabdomyosarcoma involving the orbit received chemotherapy (vincristine, actinomycin D, and cyclophosphamide) between December 31, 1968, and February 11, 1969.

In May 1969, the mother noted that the boy's left eye was sensitive to light and that there was a soft tissue swelling. Skull roentgenograms demonstrated destruction of the left orbital roof, increased density of the left frontal bone, and extension of the process into the left frontal bone. There was also clouding of the left maxillary antrum.

On May 6, 1969, he was given a course of 325 mg/m²/day of 1-acetyl-2-picolinoylhydrazine. The patient began to improve about the fourth week of therapy with decreased swelling of orbit and decreased photophobia. Repeat radiographs showed no extension of the disease and the skull roentgenogram demonstrated decreased sclerosis of the lesions. Since the clinical findings suggested that residual tumor still persisted even after more than 3 months of therapy, the dose of this drug was increased to 450 mg/m²/day orally on August 12, 1969. Definite improvement in the radiographic appear-

TABLE 1.—Summary of results of 1-acetyl-2-picolinoylhydrazine study

Tumor type	Total No. of patients	No. of patients with— Early death	Complete regression	No response or progressive disease
Rhabdomyosarcoma	10	1	1	8
Osteogenic sarcoma	5	0	0	5
Histiocytosis-X	5	0	1	4
Ewing's sarcoma	4	0	0	4
Undifferentiated sarcoma	2	0	0	2
Neuroblastoma	3	1	0	2
Fibrosarcoma	1	0	0	1
Unclassified malignant tumor	2	1	0	1
Lymphoma	2	1	0	1
Wilms' tumor	2	2	0	0
Hodgkin's disease	1	0	1	0
Totals	37	6	3	28

ance of the tumor occurred about August 26, 1969. By September 9, 1969, it was felt that a complete regression status had been achieved. The blood urea nitrogen (BUN) value, which had been normal, rose to 33 mg/100 ml on November 18, 1969. Because of this, 1-acetyl-2-picolinoylhydrazine therapy was discontinued. The patient did not receive any further therapy until he experienced hip pain with metastases to the hip and spine (T_9–T_{12}) on April 14, 1970. A bone marrow aspirate in the posterior iliac crest revealed tumor cells.

Patient No. 2

Patient No. 2 had been treated for generalized histiocytosis-X with vinblastine, cyclophosphamide, prednisone, and procarbazine at various times prior to a relapse that involved the bone marrow, skin, lymph nodes, and parotid gland. Although the ulcerating parotid lesion increased in size during the first week of 325 mg/m²/day of 1-acetyl-2-picolinoylhydrazine, all evidence of disease was gone by 28 days of treatment. This drug was continued until lymph nodes, liver, and bone marrow disease recurred at 162 days.

Patient No. 3

Stage IV Hodgkin's disease had been controlled in patient No. 3 for 2 years by various treatments including MOPP, cyclophosphamide, vinblastine, 5-fluorouracil, and CCNU. 1-Acetyl-2-picolinoylhydrazine (325 mg/m²/day) was begun when disease again recurred in the pleura, lymph nodes, liver, and bone marrow. All parameters were normal by 28 days of treatment. He continued to receive the same daily dose of 1-acetyl-2-picolinoylhydrazine for an additional 284 days with incidental complications of herpes zoster and bilateral slipped femoral capital epiphyses. His disease recurred and was exhibited as adenopathy, hepatomegaly, pruritus, fever, malaise, and night sweats.

TOXIC EFFECTS

The lethargy, drowsiness, and other neurologic abnormalities found with substantial frequency among adults (2,3) [11] were not prominent in children. Only two children reported sleepiness (one of moderate degree, one mild). One child complained of paresthesia. No other neurologic side effects were reported. Nausea and vomiting occurred in six children, one of whom also had severe abdominal pain.

Bone marrow depression was not a problem. Even though many of the children had had prior radiotherapy and extensive chemotherapy, leukopenia (partly attributed to advancing disease) was seen in one patient and mild thrombocytopenia was seen in another patient. The only serious toxic manifestation occurred in a girl with rhabdomyosarcoma who received 325 mg/m²/day of 1-acetyl-2-picolinoylhydrazine for 56 days. At that time, proteinuria and hematuria were noted with a rise in the BUN level to 85 mg/100 ml. Although the administration of the drug was discontinued, progressive renal failure ensued with uremia, hypertension, and convulsions. At autopsy, the kidneys showed the histologic appearance of chronic nephritis with striking sclerosis of virtually all glomeruli and patchy tubular atrophy. One other child had a slight rise in the BUN level to 33 mg/100 ml (patient No. 1) when 1-acetyl-2-picolinoylhydrazine was discontinued.

COMMENTS

Although the scant literature data indicate that 1-acetyl-2-picolinoylhydrazine is not very effective clinically (1–3), our experience with three good responses among 37 children treated seemed worth reporting. Wilson et al obtained no responses among 11 adults with carcinoma of the lung (3). In a pharmacologic study, no therapeutic effects were noted among 35 patients with various tumors treated with several different intravenous dose schedule regimens (2). Olson and Pratt used this drug in 24 patients and reported a response in one patient with rhabdomyosarcoma and in one patient with breast cancer (1). 1-Acetyl-2-picolinoylhydrazine possessed the advantages of oral administration and lack of bone marrow suppression. However, the low overall response rate in only three (8%) of 37 patients in this study was disappointing.

[11] *See* footnote 10.

LIST OF COMPOUNDS

1-Acetyl-2-picolinoylhydrazine: NSC-68626; CAS reg. No. 17433-31-7; Azapicyl

Actinomycin D: NSC-3053

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard

MOPP = mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-*α*-(2-methylhydrazino)-,monohydrochloride

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Adriamycin (NSC-123127) in the Treatment of Alkylator-Resistant Multiple Myeloma: A Pilot Study[1,2,3]

David S. Alberts[4] and Sydney E. Salmon[5,6]

SUMMARY

Adriamycin was given in a dose of 25–45 mg/m² of body surface area to nine patients with multiple myeloma who had failed to respond to intermittent therapy with melphalan and prednisone. Three patients had evidence of clinical remission and in two of them there was a 60% decrease in total body myeloma cell number. However, remissions were of short duration (3 months in two patients and over 10 months in another patient) and bone marrow suppression was moderately severe. Adriamycin may prove more useful in myeloma therapy when combined with melphalan or cyclophosphamide during the initial attempt at remission induction.

[Cancer Chemother Rep 59:345–350, 1975]

In patients with multiple myeloma treated with intermittent courses of melphalan and prednisone the frequency of response (defined as a 75% reduction in the M-component [monoclonal immunoglobulin] rate of synthesis as calculated from serum protein electrophoresis and fractional catabolic rate) is approximately 50%, with responding patients having a median survival time of about 3 years (1,2). Cyclophosphamide and melphalan appear to have equal activity against multiple myeloma (3) and Bergsagel et al (4) have shown that patients who become resistant to melphalan may still respond to cyclophosphamide. Procarbazine shows a low order of activity in patients resistant to melphalan but treatment with this drug has not been associated with improvement in survival (5). Most recently, several groups have been using combinations of alkylating agents including BCNU to induce remission in patients refractory to therapy with melphalan or cyclophosphamide or both (6).

We have used adriamycin, a new antitumor antibiotic with a broad spectrum of activity against carcinoma, sarcoma, lymphoma, and acute leukemia (7), to treat multiple myeloma in patients who had failed to achieve clinical remission in response to alkylating-agent/prednisone therapy. Because total body tumor cell number (8–10) was determined serially in all patients in this study, it was possible to measure small changes in plasma cell response to adriamycin therapy and to evaluate with some accuracy the oncolytic potential of adri-

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[3] Adriamycin was supplied by Cancer Therapy Evaluation, DCT, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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amycin in patients with disease which was resistant to standard therapy.

MATERIALS AND METHODS

The nine patients included in this study were treated at the Cancer Research Institute, University of California Medical Center, San Francisco, between March 1972 and May 1974. The criteria for entry into this study included an unequivocal diagnosis of multiple myeloma that had been unresponsive to at least three consecutive intermittent courses of treatment using a combination of an alkylating agent (melphalan in all nine patients, plus cyclophosphamide in four and BCNU in two) with prednisone (in all nine patients) and vincristine (in eight of nine patients) (11). At the time of initial evaluation, all patients had bone marrow plasmacytosis (> 30%), a serum M-component "spike" detected by electrophoresis, and osteolytic skeletal lesions demonstrable by roentgenograms.

The criteria for response to chemotherapy were based on the percentage reduction in tumor cell number and other evidence of clinical remission as judged by (*a*) complete relief of bone pain, (*b*) elevation of the hemoglobin value to more than 10 g/100 ml (in the untransfused patient), and (*c*) elevation of the serum albumin value to more than 4 g/100 ml. Changes in serum M-component concentration, determined by electrophoresis, were also observed and are compared in table 1 to parallel changes in tumor cell number. Response criteria gradings currently used include (*a*) excellent tumor regression—greater than 75% reduction in tumor cell number, and (*b*) partial tumor regression—greater than 50% but less than 75% reduction in tumor cell number. Patients were observed at 1–2-week intervals at which time complete blood cell counts, serum-survey-12,[7] and serum protein electrophoresis values were obtained. Total body myeloma cell number was calculated using the following formula:

$$\text{Total body myeloma cell number} = \frac{\text{Total body M-component synthetic rate}}{\text{Cellular M-component synthetic rate}}.$$

[7] Including determinations of serum phosphorus, calcium, urea nitrogen, creatinine, bilirubin, alkaline phosphatase, uric acid, total protein, and albumin.

Secretion rates of the M-component were measured by the in vitro bone marrow culture and sandwich radioimmunoassay techniques of Salmon and Smith (12).

All patients had multiple determinations of M-component secretion rates, which have remained constant (± 10%) for up to 5 years (10). Computer analysis of the data was coordinated from the Human Tumor Kinetic Center facility at the University of Arizona, Tucson, via the General Electric Mark III time-sharing computer service. The kinetics of tumor growth and tumor regression were analyzed as previously described (9, 10).

Depending upon the adequacy of the peripheral white blood cell (wbc) and platelet counts, adriamycin was given intravenously (iv) at a dosage of 25–45 mg/m² every 1–3 weeks. When the wbc count was more than 4000 cells/mm³ and the platelet count more than 125,000 platelets/mm³, adriamycin was used at a dosage of 45 mg/m². When wbc counts were between 2500 and 4000 cells/mm³ and platelet counts were between 75,000 and 125,000 platelets/mm³, the dosage level was 25 mg/m². For all patients, values for hemoglobin, wbc and platelet counts, serum protein electrophoresis (cellulose acetate technique using the Beckman Microzone cell), and total body myeloma cell number were determined at 1–2-week intervals during adriamycin therapy. Serum-survey-12 determinations were repeated at 2-week intervals and skeletal roentgenographic surveys were obtained monthly.

RESULTS

Before adriamycin therapy was started, all patients had received at least three courses of alkylating drug therapy over a period of more than 3 months. All patients had received at least two courses of high-dose intermittent therapy with melphalan and prednisone as described by Alexanian et al (11). Four patients (Nos. 4, 5, 8, and 9, table 1) in whom there had been evidence of progressive disease after 4 months of this combination therapy had been treated subsequently with at least three courses of high-dose intermittent cyclophosphamide and prednisone therapy (cyclophosphamide dose, 1.0 g/m² given by iv "push" or orally over 4 days),

TABLE 1.—Response to adriamycin therapy in patients with multiple myeloma

Patient characteristics				Response parameters											
	Prior therapy			No. of tumor cells (× 10^{12})			M-component (g/100 ml)		Hemoglobin (g/100 ml)		Serum albumin (g/100 ml)		Bone pain*	
M-component	Drugs	Duration (mos)	Total adriamycin dose (mg/m^2)	Before therapy	After therapy	Change (%)	Before therapy	After therapy	Before therapy	After therapy	Before therapy	After therapy	Before therapy	After therapy
IgGK	Melphalan, prednisone, vincristine	6	115	1.87	0.73	−61	2.9	1.3	9.1	11.0	2.7	3.2	4+	1+
IgGK	Melphalan, prednisone, vincristine	10	40	2.60	1.06	−60	5.4	3.5	10.8	12.2	2.9	2.1	4+	1+
IgGK	Melphalan, prednisone, vincristine	8	330	4.27	3.20	−25	3.1	2.7	8.3	10.0	3.4	4.0	2+	0
IgGK	Melphalan, prednisone, vincristine, cyclophosphamide	11	140	3.65	3.23	−12	6.8	6.0	7.3	9.0	2.9	2.6	2+	4+
IgAK	Melphalan, prednisone, vincristine, cyclophosphamide, BCNU	36	60	2.90	3.00	+3.4	0.4	0.4	11.9	10.3	4.4	4.3	2+	4+
IgGL	Melphalan, prednisone	24	50	1.76	2.06	+17	1.3	1.3	9.1	7.9	3.0	2.1	2+	4+
IgGL	Melphalan, prednisone, vincristine, cyclophosphamide	7	50	1.72	2.36	+37	3.0	3.8	7.5	9.7	3.5	2.5	2+	3+
IgAK	Melphalan, prednisone, vincristine, isophosphamide	16	160	3.16	4.61	+46	2.7	3.1	11.0	9.1	3.3	3.4	2+	4+
IgGK	Melphalan, prednisone, cyclophosphamide, BCNU, vincristine	10	75	2.01	4.08	+103	4.0	5.7	11.7	7.5	3.6	3.0	0	4+

* 0 = No bone pain; 1+ = minimal or occasional; 2+ = mild; 3+ = moderate; 4+ = severe.

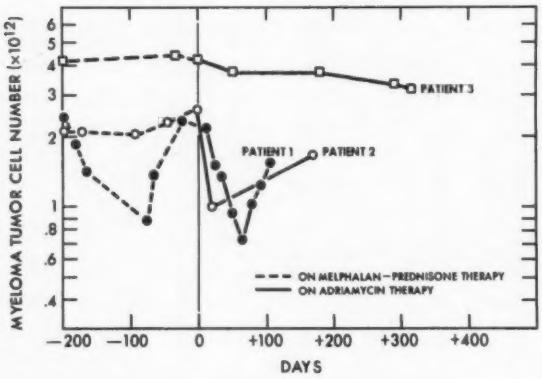

FIGURE 1.—Effects of melphalan-prednisone (.....) and then adriamycin (_____) therapy on No. of myeloma tumor cells in three responding patients (Nos. 1, 2, and 3, *see* table 1).

but again there had been no evidence of response.

In two of the nine patients (Nos. 1 and 2, table 1 and fig 1) there was a drop in tumor cell number of at least 60% during adriamycin therapy. In a third patient (No. 3, table 1 and fig 1) tumor cell number decreased by 25%. In five other patients there was significant progression in tumor size. In the remaining patient there was no change in tumor cell number during therapy.

The two patients in whom tumor cell number was reduced by more than 50% experienced almost complete disappearance of bone pain and a significant rise in hemoglobin concentration; however, both of these partial remissions lasted only 3 months. Patient No. 3, who had only a 25% decrease in tumor size while receiving adriamycin, experienced symptomatic improvement (relief of bone pain, a rise in hemoglobin of 2 g/100 ml, and a rise in albumin of 1+ g/100 ml) for 10+ months. Adriamycin treatment was discontinued in his case because of increasing edema secondary to preexisting arteriosclerotic coronary vascular disease. The remaining six patients showed no evidence of response and adriamycin therapy appeared to contribute to further clinical deterioration in some of them.

As illustrated in table 2, the patients tolerated only small doses of adriamycin because of significant and at times life-threatening leukopenia (lasting 6–20 days in the two patients achieving partial remission) and moderately severe thrombocytopenia unassociated with bleeding episodes (lasting 12–19 days in three patients).

COMMENTS

For myeloma patients who have failed to respond to melphalan-prednisone remission-induction therapy, many investigators have tried treatment with either cyclophosphamide (administered intermittently) or cyclophosphamide plus BCNU (6). Procarbazine appears to have little useful antitumor activity and vincristine has not improved the response rate when combined with melphalan and prednisone (11). In this "pilot" study, adriamycin had definite antitumor activity in three patients as determined by calculated changes in total body myeloma cell number. It is of interest that those patients who responded had lower total tumor

TABLE 2.—Toxic effects of adriamycin therapy

Patient No.	Dose (mg/m²)	Nadir wbc count (cells/mm³)	No. of days wbc count <2000 cells/mm³	Nadir platelet count (platelets/mm³)	No. of days platelet count <100,000 platelets/mm³
1	25	600	20	24,000	19
2	40	600	6	23,000	12
3	30	1600	7	178,000	0
4	45	1700	7	83,000	7
5	30	1200	14	48,000	7
6	30	550	14	20,000	60
7	30	2400	0	106,000	0
8	40	2600	0	151,000	0
9	35	2000	0	61,000	14

cell mass (per square meter of body surface area) prior to therapy than did those patients who failed to respond. Unfortunately, although all of the responses were clinically useful, two were short-lived (ie, less than 4 months). The response data have been corroborated by Alexanian et al (13) at M. D. Anderson Hospital (Houston, Tex); however, this group claims to have observed only two responses to adriamycin therapy in 21 alkylator-resistant patients with multiple myeloma.

The apparently short duration of remissions suggests that adriamycin may be of greater benefit when used in combination with alkylating drugs (melphalan or cyclophosphamide) either during initial chemotherapy or shortly after the cell mass has been significantly reduced with alkylating-agent remission-induction therapy. Conceivably, two cycle-nonspecific drugs with independent mechanisms of action on DNA (presumably intercalation for adriamycin and cross linking for alkylating agents) may have synergistic effects when used in combination. It should be emphasized that the total adriamycin dose should be limited to less than 550 mg/m^2 because with total doses above this level there is an increasing risk of irreversible cardiac toxicity (14). By combining adriamycin with alkylating agents, the true period during which adriamycin can be utilized (without exceeding the total dose limitation) could be extended.

Randomized trials evaluating combinations of cycle-nonspecific agents for remission induction are currently under way in the Southwest Oncology Group.[8] One arm of this study (protocol 7305/7306) consists of melphalan-adriamycin-prednisone remission-induction therapy. This should provide a valuable test of an adriamycin combination for remission induction in previously untreated myeloma patients.

LIST OF COMPOUNDS

Adriamycin: SC-123127

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Isophosphamide: NSC-109724; CAS reg. No. 3778-73-2; 1,3,2-oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-, 2-oxide

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-sarcolysin

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) in the Treatment of Solid Tumors in Children [1,2,3]

Jerry Z. Finklestein, Vincent Albo, Inta Ertel, and Denman Hammond [4,5]

SUMMARY

5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) was administered to 46 children with various solid tumors which were resistant to conventional therapy. Two or more courses of NSC-45388 were administered to 13 of 18 children with neuroblastoma, seven of 11 children with rhabdomyosarcoma, three of four children with Wilms' tumor, one of three children with Ewing's tumor, and six of ten children with miscellaneous neoplastic disorders. Major toxic effects included nausea, vomiting, decreased hemoglobin level, thrombocytopenia, and leukopenia. A therapeutic regimen of 200–450 mg/m²/day for 5 consecutive days can be administered safely every 22 days. Objective responses were observed in three children with neuroblastoma and in one child with rhabdomyosarcoma. This drug has minimal but definite activity as a single agent in children with advanced neuroblastoma and rhabdomyosarcoma and should be evaluated further in combination therapy.

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[3] NSC-45388 (CAS reg. No. 4342-03-4) was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[4] This study was done by the Children's Cancer Study Group (*Denman Hammond, chairman*). The following investigators participated in this study: *Vincent Albo, William Prin,* and *Salvatore Orlando,* Children's Hospital of Pittsburgh, Pa; *Wayne Borges, Allan Schwartz, Mila Pierce, James Nicklas,* and *Edda Hackl,* Children's Memorial Hospital, Chicago, Ill; *William Newton, Inta Ertel, Stella Kontras,* and *Janak Wadwa,* Children's Hospital of Columbus, Ohio; *Audrey E. Evans, Milton H. Donaldson,* and *Hyder Carim,* Children's Hospital of Philadelphia, Pa; *Myron Karon, Denman Hammond, Gussie Higgins, Carol Hyman, Jorge Ortega, Nomie Shore,* and *Kenneth Williams,* Children's Hospital of Los Angeles, Calif; *John R. Hartman, Ronald L. Chard, Jr.,* and *Irwin Bernstein,* Children's Orthopedic Hospital, Seattle, Wash; *Ruth Heyn* and *Roxie Holland,* University of Michigan, Ann Arbor; *William Krivit* and *Mark E. Nesbit,* University of Minnesota, Minneapolis; *M. Eugene Lahey* and *John F. Wilson,* University of Utah Medical Center, Salt Lake City; *Marilyn Sonley,* Princess Margaret Hospital, Toronto, Canada; *L. Gilbert Thatcher* and *Samuel R. McCreadie,* Milwaukee Children's Hospital, Wis; *Patricia Joo,* University of Wisconsin, Madison; *James A. Wolff* and *Anneliese Sitarz,* Babies Hospital, New York City, NY; *Sanford Leikin* and *Nasser Movassaghi,* Children's Hospital of Washington, DC; *Martin Klemperer,* University of

5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) is a synthetic agent that is markedly effective against a variety of solid tumor systems.[6] This drug has been used in various patients with solid tumors. Objective antitumor responses were initially reported in patients with cancer of the kidney, testicular tumors, rhabdomyosarcoma, leiomyosarcoma, carcinoma of the lung, hepatoma, and mesothelioma.[7] These initial results prompted the Children's Cancer Study Group to undertake a study of NSC-45388 in children with acute leukemia and solid tumors. The experience in the treatment of advanced acute lymphocytic leukemia of children has been previously reported (1). This report will document our experience in determining the activity of intravenous (iv) NSC-45388 in children with various solid tumors.

MATERIALS AND METHODS

Children with various solid tumors were eligible for entry into the study if they had measurable disease and were resistant to conventional antitumor chemotherapy. All patients were refractory to cyclophosphamide and vincristine.[8] Children with rhabdomyosarcoma, Wilms' tumor, hepatic carcinoma, and undifferentiated sarcomas were also resistant to actinomycin D (NSC-3053). All patients had advanced disease and they ranged in age from 1 to 15 years.

NSC-45388 was reconstituted with sterile water, diluted with 5% dextrose and water, and infused iv over a period of 15–20 minutes. This was done to avoid the pain which can be experienced with rapid injection of the undiluted agent (2). The solution was usually administered immediately after reconstitution although it is stable at room temperature for at least 12 hours.

Patients received an initial course of NSC-45388 of at least 250 mg/m²/day iv for 5 consecutive days and whenever possible, a second 5-day course of 200–400 mg/m²/day was administered 12–26 days later. Subsequent courses and doses were adjusted for each patient.

Physical examinations and hematologic evaluations were required at least once prior to the initiation of each course of therapy. Bone marrow aspirations were optional prior to the start of therapy. Children with solid tumors in whom regression of index lesions was greater than 50% in all measurable dimensions were considered to have a partial response and children in whom all measurable disease had completely disappeared were considered to have a complete response. Patients were removed from study when progressive disease was evident although if possible, two courses of therapy were administered prior to their removal from the protocol.

RESULTS

Forty-six children received at least one 5-day course of NSC-45388 (table 1). Two or more courses of the drug were administered to 13 of 18 children with neuroblastoma, seven of 11 children with rhabdomyosarcoma, three of four children with Wilms' tumor, one of three children with Ewing's tumor, and six of ten children with miscellaneous neoplastic disorders.

Hematologic effects were observed with a decrease in hemoglobin level to 8 g or less in 12 children, a decrease in the platelet count to less than 75,000 platelets/mm³ in 18 children, and a decrease in the total white blood cell (wbc) count to 2900 cells/mm³ or less in 22 children. The nadir for leukopenia occurred between 2 and 25 days after the initiation of a course; the mean time was 10 days. Five

Rochester, NY; *Denis R. Miller*, Cornell Medical Center, New York, NY; *Robert Baehner*, Indiana University, Indianapolis; *J. Mavis Teasdale*, University of British Columbia, Vancouver, Canada; *Jerry Z. Finklestein, Lance Sieger, Lowell Greenberg*, and *Victor Medrano*, Harbor General Hospital, Torrance, Calif; *Leonard Vitale*, New Jersey College of Medicine, Newark.

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[8] Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

TABLE 1.—Patient information

Patient No.	Diagnosis	Sex	Age (yrs)	Duration of disease prior to treatment with NSC-45388 (mos)	Dose schedule mg/m²/day	Dose schedule Days	Wbc at start of course (cells × 10³/mm³)	No. of days on study	Response	Reason for removal from study*
1	Neuroblastoma	F	3 7/12	14	237	1–5	4.5	72	Partial	PD
					350	15–19	7.2			
					470	30–34	5.6			
					470	55–60	3.4			
2	Neuroblastoma	M	8 1/12	13	450	1–5	11.0	102	No	PD
					450	40–44	3.7			
					450	54–58	4.8			
					450	68–72	5.0			
					450	82–86	5.8			
3	Neuroblastoma	M	3 1/2	21	250	1–5	2.5	16	No	+
4	Neuroblastoma	M	7	6	250	1–5	6.1	28	No	PD
					250	15–19	3.7			
5	Neuroblastoma	M	3	11	250	1–5	2.8	30	No	+
					450	18–22	—			
6	Neuroblastoma	F	2	21	250	1–5	1.5	25	No	PD
					250	22–26	—			
7	Neuroblastoma	F	6 1/2	12	250	1–5	7.0	38	No	PD
					250	24–28	9.4			
8	Neuroblastoma	F	6 1/2	13	250	1–5	—	10	No	–
9	Neuroblastoma	M	11 9/12	17	250	1–5	5.3	48	No	PD
					250	15–19	—			
10	Neuroblastoma	M	4 1/2	9	250	1–5	1.7	33	No	PD
					250	15–19	3.4			
11	Neuroblastoma	M	4	56	250	1–5	—	40	No	PD
					250	35–39	—			
12	Neuroblastoma	M	2 7/12	4	250	1–5	21.9	9	No	+
13	Neuroblastoma	M	1	19	200	1–5	2.1	13	No	+
14	Neuroblastoma	F	5 4/12	7	250	1–5	11.3	52	No	+
					400	15–19	5.6			
					400	29–33	6.0			
15	Neuroblastoma	F	5	5	225	1–5	5.6	11	No	+
16	Neuroblastoma	M	4	6	250	1–5	4.0	62	Partial	PD
					250	15–19	4.9			
					400	29–33	4.2			
					400	39–43	2.2			
17	Neuroblastoma	F	2 9/12	8	400	1–5	5.0	71	Partial	PD
					200	35–39	2.0			
					200	49–53				
18	Neuroblastoma	F	1 7/12	7	250	1–5	3.7	52	No	PD
					250	13–17	2.2			
					250	26–30	—			

TABLE 1.—Patient information—Continued

Patient No.	Diagnosis	Sex	Age (yrs)	Duration of disease prior to treatment with NSC-45388 (mos)	Dose schedule mg/m²/day	Dose schedule Days	Wbc at start of course (cells × 10³/mm³)	No. of days on study	Response	Reason for removal from study*
19	Rhabdomyosarcoma	F	14 1/2	8	250	1–5	5.4	13	No	PD
20	Rhabdomyosarcoma	M	2 10/12	15	250	1–5	4.3	35	No	PD
21	Rhabdomyosarcoma	M	13 8/12	10	250	1–5	3.7	26	No	+
22	Rhabdomyosarcoma	F	15	17	250 250	1–5 15–19	2.1 0.7	27	No	+
23	Rhabdomyosarcoma	M	13	4	250 250	1–5 15–19	5.9 7.2	27	No	PD
24	Rhabdomyosarcoma	F	6 1/2	22	250	1–5 19–23	6.3 4.1	35	No	+
25	Rhabdomyosarcoma	M	1 4/12	16	250 400 250 250 250	1–5 18–22 33–37 46–50 68–72	2.7 5.1 2.7 3.8 6.8	71	Partial	PD
26	Rhabdomyosarcoma	M	8 5/12	42	250 400 400	1–5 17–21 31–35	10.7 7.4 4.9	45	No	–
27	Rhabdomyosarcoma	M	14	4	250 250	1–5 15–19	11.1 —	28	No	PD
28	Rhabdomyosarcoma	F	11	11	250 400 400	1–5 15–19 29–33	2.6 3.8 —	35	No	PD
29	Rhabdomyosarcoma	F	5	5	250	1–5	11.8	16	No	+
30	Wilms' tumor	M	1 3/12	11	250 450 450 450	1–5 15–19 29–33 43–47	5.5 6.0 7.6 3.4	56	No	+
31	Wilms' tumor	F	1	11	250	1–5	11.0	14	No	+
32	Wilms' tumor	M	2 9/10	32	250 400	1–5 20–24	8.9 —	25	No	PD
33	Wilms' tumor	M	8 10/12	21	250 250 250	1–5 18–22 32–36	8.7 7.9 8.0	45	No	PD
34	Ewing's tumor	M	11 2/12	5	250	1–5	7.8	14	No	+
35	Ewing's tumor	F	6 1/2	85	250 250	1–5 15–19	9.0 7.9	39	No	+
36	Ewing's tumor	M	14 7/12	13	250	1–5	2.4	14	No	PD

TABLE 1.—Patient information—Continued

Patient No.	Diagnosis	Sex	Age (yrs)	Duration of disease prior to treatment with NSC-45388 (mos)	Dose schedule mg/m²/day	Dose schedule Days	Wbc at start of course (cells × 10³/mm³)	No. of days on study	Response	Reason for removal from study*
37	Hepatic carcinoma	M	12	18	250	1–5	4.6	115	No	PD
					400	22–26	5.5			
					400	43–47	4.7			
					400	54–58	6.3			
					400	75–79	5.9			
					400	86–90	6.1			
					400	111–115	4.9			
38	Undifferentiated sarcoma	M	16 11/12	33	250	1–5	6.1	35	No	PD
					250	22–26	4.7			
39	Undifferentiated sarcoma	M	3	11	250	1–5	—	9	No	+
40	Malignant mesenchymoma	M	15	78	250	1–5	3.0	57	No	PD
					250	29–33	4.3			
					400	43–47	11.1			
41	Osteogenic sarcoma	F	14 11/12	17	250	1–5	—	2	No	◆
42	Medulloblastoma	F	7 1/2	8	250	1–5	9.6	23	No	+
					400	15–19	—			
43	Reticulum-cell sarcoma	M	4 1/12	3	250	1–5	1.0	17	No	+
					400	13–17	3.6			
44	Embryonal carcinoma	M	4	16	250	1–5	8.8	10	No	−
45	Embryonal carcinoma	F	1 2/12	5	250	1–5	5.2	14	No	PD
					250	11–15	12.6			
46	Embryonal carcinoma	F	2 11/12	23	250	1–5	9.9	18	No	PD

*PD = progressive disease; + = died; − = removed from study by parents' request; ◆ = removed from study by physician's request.

children demonstrated severe leukopenia with a total leukocyte count of 1000 cells/mm³ or less and in two of these patients a complicating infection occurred. In 18 of the 53 followup courses, therapy was delayed after 15 days due to hematologic toxic effects. Twenty-two children required supportive transfusions with sedimented cells. However, in all cases the peripheral blood counts recovered; this allowed the continuation of drug therapy for subsequent courses. All children experienced some nausea and vomiting secondary to the administration of NSC-45388. This complaint was usually controlled with antiemetics. Four patients had mild elevations in serum glutamic oxaloacetic transaminase values without other evidence of liver disease. The flu-like symptoms which have been reported by other investigators (2) were not observed. Eight children required systemic antibiotic therapy; two children died of septicemia.

Responses were seen in three children with neuroblastoma who received two or more courses of therapy and in one child with rhabdomyosarcoma who also received two or more courses of therapy. Measurable regression

of tumor was noted in all patients who responded after the first course of therapy. None of the remaining patients demonstrated an objective response.

The clinical courses given to children with neuroblastoma who responded are described. (*a*) A 3½-year-old girl with widespread stage IV disease had regression of a buttock lesion and a right lower quadrant mass. In addition, there was healing of lesions in the frontal and vertebral bones. The maximum response occurred after the administration of a third course of therapy and lasted for approximately 6 weeks during continuous therapy. (*b*) A 4-year-old boy demonstrated marked regression of a mass in the left neck and almost complete clearing of a widespread infiltration of the bone marrow after two courses of therapy. (*c*) A 2½-year-old girl had complete disappearance of cervical and inguinal adenopathy, regression of liver metastases, and partial clearing of tumor cells in the bone marrow after she received three courses of therapy.

The response in the child who had rhabdomyosarcoma occurred in a 1-year-old boy who demonstrated regression of palpable tumor masses in the anterior-posterior neck areas, including involvement of the mid-sternocleidomastoid muscle. Tumors as large as 4 × 5 cm became nonmeasurable. Other measurable lesions became smaller (less than 1½ cm in diameter). Maximum regression occurred after he received two courses of therapy.

Patients remained in the study from 2 to 115 days. Three patients (Nos. 8, 26, and 44) were removed from the study at the request of the parents and one patient (No. 41) was removed from the study by her physician in favor of an alternate treatment program. Seventeen children died during the study because of progressive disease. The remaining patients were removed from the study upon documentation of progressive disease.

DISCUSSION

The schedule adopted in our study was similar to the one used by Luce et al (2) in which NSC-45388 was given to 171 evaluable patients at a dose of 200–500 mg/m²/day for 5 days and repeated at 3-week intervals. In that study, five of 23 patients with sarcomas and 21 of 121 patients with melanomas demonstrated a response, whereas no responses were seen in eight patients with lung carcinoma, four patients with carcinoma of the colon and breast, and two patients each with Hodgkin's disease, hypernephroma, Wilms' tumor, and embryonal carcinoma of the testis. Frei et al (3) used a similar program and observed an objective response in ten of 18 patients (56%) with Hodgkin's disease, one of which was complete. Four of 15 patients with lymphosarcoma and one patient with mycosis fungoides responded. None of 13 patients with reticulum-cell sarcoma responded to therapy. Kingra et al have also studied NSC-45388 for the treatment of malignant tumors other than melanoma using a dose level of 4.5 mg/kg/day for 10 days (4). Objective tumor shrinkage occurred in four of 44 patients (9%) with lung cancer and "dramatic benefits" were noted in three of five patients with primary brain tumors. The results reported in our study revealed that NSC-45388 shows some activity in children with neuroblastoma and rhabdomyosarcoma. Furthermore, NSC-45388 is tolerated at a dose of 200–450 mg/m²/day × 5, every 14–28 days. The optimal schedule appears to be at 22-day intervals since 45 of the 53 repeated courses were administered within that time span.

Recent investigations (5–7) have revealed that NSC-45388 may act in adults as an additive when combined with vincristine, BCNU, cyclophosphamide, or adriamycin.[9] Recently, we have found that combination therapy with NSC-45388, vincristine, and cyclophosphamide results in an improved response rate in children with widespread metastatic neuroblastoma (8).

Our study has defined the toxic effects of NSC-45388 in children and has revealed that a therapeutic regimen of 200–450 mg/m²/day for 5 days can be administered every 22 days. One must conclude that NSC-45388 has minimal but definite activity as a single agent in children with advanced solid tumors. The drug should be evaluated further in combination therapy.

[9] BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.
Adriamycin: NSC-123127.

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Combined Chemotherapy in Childhood Rhabdomyosarcoma [1,2]

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SUMMARY

Twenty-two children with rhabdomyosarcoma were treated with combination chemotherapy using vincristine, actinomycin D, and cyclophosphamide for varying time periods. Chemotherapy, usually combined with radiation therapy, was given to early stage patients after they had had a complete or partial tumor resection. Three of nine patients who had widespread metastatic (stage III) disease at initiation of therapy had complete tumor regression and four of nine had partial tumor regression. However, the median durations of response and survival were brief (3 and 8 months respectively). Five patients with localized resectable (stage I) disease have survived without evidence of tumor recurrence from 33 to 69 months after their initial diagnosis. Of eight patients with incompletely resected regional (stage IIB) disease, one has survived without disease recurrence for 36 months, though the median survival time of the remaining seven patients was 14 months. In stage IIB patients the duration of response was proportional to the duration of chemotherapy which suggests that chemotherapy should be given for at least 18–24 months.

[Cancer Chemother Rep 59:359–365, 1975]

Several investigations have indicated that combination chemotherapy administered to children who have rhabdomyosarcoma is temporarily effective in controlling widespread metastatic disease (1–6) and that combination chemotherapy given in conjunction with surgery and radiation therapy can greatly prolong the survival of children who have localized disease at the time of diagnosis (3,4,6–9).

Although a high percentage of children with widespread metastatic rhabdomyosarcoma have shown a complete or partial response to combination chemotherapy in previous studies, the median duration of response has usually been less than 9 months, with the percentage of long-term survivors with metastatic disease being less than 1% (3,4,9,10). In contrast, much longer durations of response (as well as long-term survivals) have been achieved in children with localized or regional disease when the combined modalities of surgery, radiation therapy, and chemotherapy (3,4,6–9) are utilized.

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This study reports further results of the treatment of rhabdomyosarcoma in children utilizing the combination chemotherapy of vincristine, actinomycin D, and cyclophosphamide (VAC).[a] Our group includes a high proportion of children who had widespread metastatic disease at the time of diagnosis.

METHODS

This study accepted new entries from June 1968 through November 1972. All surviving patients have been observed for more than 33 months. Initially, only patients with widespread metastatic rhabdomyosarcoma were eligible for this study, but later patients with nondisseminated disease were also admitted. None of the patients had received prior chemotherapy.

Clinical Staging

The following clinical staging was used (4):

Stage I—localized disease, completely resected tumor;

Stage II—regional disease, adjacent structure, or local or regional lymph nodes involved;

A—completely resected tumor;
B—incompletely resected or nonresected tumor;

Stage III—generalized disease with distant metastases.

Of 26 patients who were registered in this study, 11 had widespread metastatic disease at the time of diagnosis. The other 15 patients included six with stage I disease, none with stage IIA disease, and nine with stage IIB disease.

Chemotherapy

The chemotherapy regimen utilized was as follows:

a. vincristine, 1.5 mg/m²/wk intravenously (iv) for 6 weeks, then every 2 weeks for 6 weeks;
b. cyclophosphamide, 300 mg/m²/wk iv for 6 weeks, then every 2 weeks for 6 weeks;
c. actinomycin D, 0.4 mg/m²/wk iv for 6 weeks.

Extension of this treatment plan after 12 weeks was optional but 12 patients received an extension of treatment using either all three drugs or just vincristine and cyclophosphamide. These extensions ranged from 3 additional months to 2 years of total therapy. Patients who received therapy extension after a year received drugs at more widely spaced intervals during the second year.

Blood counts were taken prior to the administration of each dose of chemotherapy, and chemotherapy was withheld if the total white blood cell (wbc) count fell below 2000 cells/mm³, the absolute granulocyte count fell below 1500 cells/mm³, or the platelet count fell below 100,000 platelets/mm³.

Radiation Therapy

Each investigator could decide whether or not to use radiation therapy in addition to surgery and chemotherapy. Eight patients with stage I or II disease received radiation therapy which was administered to the tumor site either preceding or concomitant with chemotherapy and after initial surgery. If stage III patients received palliative radiation therapy to one or more tumor areas, radiation therapy was not given to lesions which were being evaluated for response to chemotherapy.

Response

A complete response was indicated by a complete regression of all apparent tumor, including lesions noted on roentgenograms. A partial response was defined as a greater than 50% but less than 100% regression of at least one measurable lesion in the absence of progression of any other lesion. No response was indicated by a less than 50% decrease in at least one measurable tumor lesion.

RESULTS

Of the 26 patients registered in this study,

[a] Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).
Actinomycin D: NSC-3053.
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate.
All drugs used in this study were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

22 were evaluable. Tables 1 and 2 summarize the data on evaluable patients in relation to age at diagnosis, primary site of tumor, histologic type, sites of metastases, extent of resection, amount of radiation therapy employed, duration of VAC therapy, degree and duration of response to therapy, interval from termination of VAC therapy to recurrence or increase of tumor, and duration of survival.

Patients With Disseminated Disease (Table 1)

Of 11 patients with stage III disease at the time of chemotherapy initiation, nine were evaluable. Two inevaluable stage III patients included one who was lost to followup 2 weeks after initiation of therapy, and one who had flaccid paralysis after 1 week of chemotherapy. The latter patient's paralysis was thought to be due to disseminated disease but he was removed from the study because of the possibility of vincristine toxicity.

Of nine evaluable stage III patients, three patients had complete response, four patients had partial response, and two patients had no response. After having received only one dose of triple chemotherapy, one of the patients who showed no response died early due to disease. The median duration of response in stage III patients was 3 months and the median duration of survival was 8 months. Recurrence or progression of disease developed while VAC therapy was being given to five patients.

Patients Without Metastatic Disease (Table 2)

Of the six patients who were classified as having stage I disease at the time of diagnosis, one was lost to followup after 3 weeks of therapy and was therefore inevaluable. The other five stage I patients are long-term survivors. Three have had no recurrence and are alive at 34, 69, and 38 months respectively. Two patients have had local disease recurrences and second tumor resections, again judged to be complete. In one child the local recurrence was diagnosed 3 months after initial surgery, and radiation therapy and chemotherapy were initiated only after the second resection was performed. This child has now been alive for 33 months without evidence of disease. The other child received only 3 months of chemotherapy after initial surgery, with local recurrence of disease occurring at 15 months. The second resection was followed by radiation therapy and additional chemotherapy, and this child has been alive for 60 months without evidence of disease.

All nine patients with stage IIB disease had gross residual disease remaining after surgery. Of these nine patients, one was not evaluable after being lost to followup at 6 weeks. Of the remaining eight patients, one is alive with no evidence of tumor at 36 months after diagnosis. This patient was treated with VAC for 24 months. The remaining seven patients were not long-term survivors, although initially, four had complete responses and three had partial responses. The median duration of response in these seven patients was 9 months and the median duration of survival was 14 months. In five of these patients (each treated for 6 months or less with VAC) there was disease recurrence or disease progression within 3–4 months after VAC was discontinued.

Chemotherapeutic Toxicity

Vomiting was reported in 12 patients and occurred either immediately or within several hours after chemotherapy was administered. Alopecia occurred early during therapy but improved during extended therapy.

Depressed deep-tendon reflexes were common. Two patients had transient lower extremity paresthesias which necessitated the discontinuance of vincristine for 1–2 weeks. Two patients had moderately severe constipation secondary to vincristine administration.

One patient exhibited severe buccal mucositis during the administration of combination chemotherapy and radiation therapy for a tumor originating in the maxillary sinus. One patient had hemorrhagic cystitis which required the discontinuance of cyclophosphamide; however, this patient had also received radiation therapy to the bladder.

Granulocytopenia of less than 1500 cells/mm^3 (absolute granulocyte count) occurred during therapy in 11 of the patients and necessitated a delay in drug administration for usually not more than 1 week. Life-threatening neutro-

TABLE 1.—Results of therapy with VAC in children with widespread metastatic rhabdomyosarcoma (stage III)

Patient age (yrs)	Primary tumor site	Histologic type	Sites of metastases	Surgery	Radiation therapy to—	Duration of VAC therapy (mos)	Response	Duration of response (mos)	Recurrence	Survival from diagnosis (mos)
12	Left calf	Alveolar	Left femoral nodes, left testicle, thorax, lungs, liver	Biopsy	Mediastinum, 4000 rads	6	Complete	3	*	12
12	Pharynx	Embryonal	Neck nodes, brain stem	Biopsy	Left face, neck, pharynx (4000 rads); midcranial fossa (4950 rads)	6 (begun 4 mos after diagnosis)	Complete	3	*	13
7	Right chest wall	Undifferentiated	Nodes, lungs, marrow	Biopsy	Right chest, 3000 rads	6	Partial	3	*	12
7	Pelvis	Embryonal	Liver (5 mos after surgery)	Cystectomy, hysterectomy	Pelvis with local recurrence at 5 mos	2 1/4 (begun 5 mos after surgery)	No response			7
2 1/2	Left ear	Embryonal	Lungs, skull, femur, cervical nodes	Biopsy	Left ear (5000 rads); spine, hip (3000 rads)	3 1/2	Partial	2	*	6
15	Scrotum	Embryonal	Lungs, bones, cord compression T_4 (2 mos after initial surgery)	Removal of scrotal mass	Site of cord compression	3 (begun 2 mos after initial surgery)	Partial	2 1/2	†	8
5	Left lower leg	Undifferentiated	Left inguinal nodes	Node biopsy	Left leg (begun 3 mos after VAC)	3	Partial	3	†	11 1/2
14	Perineum	Embryonal	Inguinal nodes, marrow	Biopsy	Pelvis (3200 rads); perineum (2500 rads)	6	Complete	6	*	8
10	Nasopharynx vs orbit	Undifferentiated	Multiple bones, marrow	Biopsy	Left orbit, left face, pharynx (5300 rads) (prior to chemotherapy)	1 dose only	No response			2 1/2

*Recurrence or disease progression prior to stopping VAC.
† Increasing disease within 1 mo after stopping VAC.

TABLE 2.—Results of treatment of children with rhabdomyosarcoma without distant metastases (stages I and II)

Disease stage	Patient age (yrs)	Primary tumor site	Extension	Histologic type	Surgery	Radiation therapy to—	Duration of VAC therapy (mos)	Response	Duration of response (mos)	Recurrence (mos after VAC stopped)	Survival from diagnosis (mos)
I	2 9/12	Left forearm	None	Alveolar	Complete excision	Forearm, 7000 rads	22	Complete	34*		34*
I	2	Right upper arm	None	Embryonal	Amputation	None	25	Complete	69*		69*
I	4/12	Right neck	None	Embryonal	Complete excision	None	1 1/2	Complete	38*		38*
I	2 10/12	Left temporal area	None	Embryonal	Excision of mass initially and of local recurrence at 3 mos	None	3 (begun after recurrence)	Complete	33*		33*
I	4 6/12	Left spermatic cord	None	Undifferentiated	Left radical orchiectomy initially; resection of local recurrence at 15 mos	None initially; local recurrence, 5000 rads	3 (initially); 9 mos of cyclophosphamide after recurrence	Complete	40 (from recurrence)	12	60*
IIB	3	Right maxillary sinus	Right cheek	Embryonal	Partial resection	Right face, 6000 rads	24	Complete	36*		36*
IIB	15	Abdomen	Diffuse in abdomen	Undifferentiated	Partial resection	None	5	Partial	7	3	9
IIB	3 6/12	Nasopharynx	External auditory canal	Embryonal	Partial resection	Nasopharynx, 5000 rads	6	Complete	9	2	12
IIB	4 6/12	Paraspinous muscles	Vertebrae, epidural space	Embryonal	Partial resection, cord decompression	Paraspinous area, 2550 rads	3	Complete	9	4	14
IIB	2 6/12	Paraspinous muscles	Epidural space	Embryonal	Partial resection, cord decompression	Paraspinous area, 4000 rads	12	Complete	17	5	17
IIB	4	Posterior pharynx	External auditory canal	Alveolar	Partial resection	Pharynx, incomplete course (begun after 3 mos VAC)	3	Partial	4	2	6
IIB	14	Abdomen	Diffuse in abdomen	Embryonal	Biopsy	None	3	Partial	5	3	21
IIB	13	Prostate	Bladder	Alveolar	Biopsy	Perineum (4150 rads); medial pelvis, paraaortic nodes (3515 rads)	12	Complete	24	12	31

*Alive and free of disease.

penia was not encountered, and no chemotherapy-related sepsis was seen. No deaths were felt to be drug related.

DISCUSSION

These results suggest a very poor prognosis for the child with widely disseminated rhabdomyosarcoma, since there were no survivors beyond 13 months in the nine patients who had disseminated disease at the time therapy was initiated. Although triple therapy produced a complete or partial response in seven of the stage III patients, the duration of response was brief.

Conversely, five long-term survivors with stage I disease (all alive without evidence of disease at more than 33 months) demonstrate the excellent results which can be obtained by using combined treatment modalities in children who have localized rhabdomyosarcoma. When tumor recurrence is confined to local recurrence, long-term survival may still be achieved, as evidenced by the two stage I patients whose local recurrences were controlled. One of the stage I patients is unusual in that she has done well in spite of her young age at the time of diagnosis (4 months). Patients under 1 year of age with rhabdomyosarcoma have been noted to do poorly in some series (10,11). It should be noted that in the stage I patients there was not a disproportionate number of patients with primary tumors in sites previously shown to have a favorable prognosis (3).

Patients with gross residual disease which remains after surgery have been shown previously to have a poor prognosis as compared to those with only microscopic residual disease (6). All eight patients with stage IIB disease in the present series had gross residual disease remaining after surgery. Although one of the patients is alive without disease 36 months after diagnosis, the median duration of survival of the remaining seven patients was only 14 months.

All five responders with stage IIB disease who were treated for 6 months or less with VAC had recurrence or progression of disease within 4 months after therapy was discontinued, suggesting that chemotherapy should have been given for a longer period. The two stage IIB patients receiving chemotherapy for 1 year showed response durations of 17 and 24 months respectively. This suggests that chemotherapy should have been extended beyond a year. The remaining stage IIB patient who is currently free of disease at 36 months after diagnosis received 24 months of VAC, perhaps suggesting that chemotherapy should be extended for a minimum of 2 years. It has previously been shown that most local recurrences and distant metastases occur within 2 years (3,9,10). Similar suggestions about duration of treatment for stage IIB disease have been made by other investigators (7,9).

It should be noted that the amount of radiation therapy administered to the primary tumor in some of our patients with nonmetastatic disease was less than the presently recommended dose of 5000–6000 rads, local tissue tolerance permitting. With high-dose radiation therapy and with combination chemotherapy for 2 years' duration, higher percentages of long-term survivors with incompletely resectable rhabdomyosarcoma are now being reported (7,12).

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Chemotherapeutic Management of Bronchogenic Carcinoma: Trials With NSC-140117, a New Methanesulfonic Acid Ester of Aminoglycol [1,2]

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SUMMARY

A new drug, NSC-140117, was used in the management of 15 patients who had late bronchogenic carcinoma and were unable to receive surgical or radiotherapeutic treatment. Good responses in the form of 90% shrinkage in the size of the lesions were encountered in nine patients (60%). Response is still being maintained from 6 months to 1.5 years after the start of therapy. No toxic effects, including hematopoietic toxicity, were encountered.

[Cancer Chemother Rep 59:367–370, 1975]

Recently available chemotherapeutic agents have not been consistently effective in palliating bronchogenic carcinoma. For this reason regimens are planned merely to slow the rate of growth of tumors and to make the lifetime remaining to every patient as comfortable and useful as possible.

This paper describes a trial of a new antitumor methanesulfonic acid ester of aminoglycol, 1-propanol, 3,3'-imino-, dimethanesulfonate (ester), *p*-toluenesulfonate (NSC-140117), in the management of bronchogenic carcinoma. This compound has the same alkylating group (methanesulfonyl) as busulfan.[7] However, unlike busulfan, NSC-140117 is water soluble and possesses a strong potency against a wide spectrum of tumors (1–3). The preparation was supplied in two forms: 100-mg ampules or 10-mg tablets. The dried material, though stable when dry, should be kept at 4° C. It is freely soluble in water. Each ampule must be dissolved in 10 ml of distilled water and should be rapidly injected directly intravenously (iv).

The chemotherapeutic effect of this drug on malignant lymphoma and chronic myeloid leukemia has already been reported (4,5). The chemical and screening data have been documented by El-Merzabani and Sakurai (1–3).

MATERIALS AND METHODS

Fifteen patients with progressive unresectable lung cancer who were ineligible for current radiotherapeutic protocols because of the extent of their disease were included in this study. There were 13 men and two women. Ages ranged from 40 to 59 years. All were referred to the Medical Oncology Department, Cancer Institute, Cairo, Egypt, for chemothera-

[1] Received June 4, 1974; revised Aug 29, 1974; accepted Sept 24, 1974.

[2] NSC-140117: CAS reg. No. 32784-82-0; 1-propanol, 3,3'-iminodi-, dimethanesulfonate (ester), *p*-toluenesulfonate; No. 864T. Supplied by Yoshitomi Pharmaceutical Co., Tokyo, Japan.

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[7] Busulfan: NSC-750; CAS reg. No. 55-98-1; 1,4-butanediol, dimethanesulfonate.

peutic management. Nine patients had regional disease only while six patients also had distant metastases (table 1). Eleven patients were ambulatory, two were semiambulatory, and two were bedridden.

Diagnosis was reached by history, clinical examination, radiology, bronchoscopy, cytology, and in two cases, open surgery. Every patient was maintaining a reasonable state of nutrition and had a hematologic status that did not preclude vigorous cytotoxic chemotherapy, ie, all patients had a leukocyte count greater than 4000 cells/mm^3 and a platelet count greater than 100,000 cells/mm^3. All patients had measurable malignant lesions and adequate renal function.

NSC-140117 was given to every patient as an iv injection of 100 mg daily for 5 consecutive days. This course was repeated monthly for 3 months and then a maintenance dose of 100-mg tablets was given twice a week. A complete peripheral blood cell count was done once weekly.

Re-evaluation of the clinical condition was carried out every 4 weeks and included case history, physical examination, and measurement of indicator lesions. Objective improvement as assessed radiologically was defined as a measurable reduction in the size of the indicator lesion. Patients were considered to have progressive disease if any lesion increased in size or if a new lesion developed.

RESULTS

All patients had unresectable bronchogenic carcinoma with a history of cough, hemoptysis, weight loss, and anorexia. Duration of symptoms varied from 2 to 6 months before entry into the study. All men were heavy smokers;

TABLE 1.—Patient information

Patient No.	Age, sex	Pathology	Lobe site	Extrathoracic spread	Response	Site of response	Duration of response (mos)
1	42, M	Squamous cell	Left upper	Left vocal cord paralysis	None	—	—
2	50, M	Squamous cell	Left upper	—	Good	Local lesion	6
3	55. M	Squamous cell	Right upper	Right hemiplegia	Good	Local lesion and hemiplegia	9
4	46, M	Squamous cell	Left upper	—	Good	Local lesion	8
5	52, M	Squamous cell	Left upper	Anterior chest mass	Good	Local lesion and chest mass	12
6	59, M	Squamous cell	Left upper	—	Good	Local lesion	18
7	47, M	Squamous cell	Right lower	Liver	None	—	—
8	40, M	Squamous cell	Right lower	—	Good	Local lesion	8
9	53, M	Squamous cell	Right lower	—	Died	—	—
10	56, M	Squamous cell	Right lower	Liver	None	—	—
11	50, M	Oat cell	Left lower	—	Good	Local lesion	14½
12	54, M	Oat cell	Left lower	—	Died	—	—
13	51, M	Adenocarcinoma	Left lower	—	Good	Local lesion	9
14	58, F	Adenocarcinoma	Left lower	—	Good	Local lesion	8
15	50, F	Oat cell	Right upper	Superior vena caval obstruction	None	—	—

neither of the women was a smoker. Clinically, there was emaciation in ten of 15 patients and clubbing in nine patients. There were signs of superior vena caval obstruction in one woman. One man had left vocal cord paralysis and one patient had right-sided hemiplegia. Liver involvement with metastases was encountered in two patients. One patient had an anterior chest wall mass.

Radiologically, using both posteroanterior and lateral views, nine patients had left-sided and six had right-sided carcinoma (seven of the upper lobe and eight of the lower lobe). There were radiologic findings of hilar involvement in 11 of the 15 patients. Bronchoscopy showed either the tumor or secondary evidence of tumor indicated by fixation of the bronchi, blunting of the tracheal carina, or displacement of a bronchus.

Histopathology was reached by tissue biopsy: through bronchoscopy in 11 patients, open surgery biopsy in two, liver biopsy in one, and biopsy from anterior chest wall mass in one. Repeated cytology revealed squamous cell carcinoma in ten patients, oat cell carcinoma in three, and adenocarcinoma in two (table 1).

The effect of chemotherapy was both subjective as well as objective. Subjective improvement began to appear during the first week after the first course of treatment. There was a sense of well being with an increase in appetite and a gradual increase in weight.

Objective improvement was followed clinically as well as radiologically every 4 weeks. Nine patients, eight men and one woman, responded by gradual diminution in size of the lesion (50% shrinkage in the first month, 70% in the second month, and 90% in the third month), with complete disappearance of hilar glandular involvement and extrathoracic manifestations. Thus, the right-sided hemiplegia gradually improved and finally disappeared. The anterior chest wall mass regressed completely. Although patients are still receiving maintenance therapy, there is still a residual mass in the affected lungs. Cough ameliorated, hemoptysis stopped, and clubbing disappeared.

Responses occurred in six patients with squamous cell carcinoma, two patients with oat cell carcinoma, and one patient with adenocarcinoma. Of the six remaining patients, two showed a partial response with 50% shrinkage after the first course. These included one patient with squamous cell carcinoma and one patient with adenocarcinoma. Followup of these two patients showed an increase in the size of the lesions in spite of chemotherapy. Four patients showed no response and their conditions gradually progressed. Two of them died 1 month after starting treatment, one from acute myocardial infarction with electrocardiographic findings and the other from a severe attack of hemoptysis. Neither showed any response to drug therapy (table 1).

No toxic effects from drug administration were encountered. The hematologic picture was not affected, including hemoglobin, red blood cells, white blood cells, and platelets.

CASE REPORT

Patient No. 6 was a 59-year-old policeman. He had inoperable left-sided bronchogenic squamous cell carcinoma and was referred to us from a thoracic surgeon. There was hilar involvement as well as pericardial infiltration. He received the course of NSC-140117 as mentioned previously with gradual improvement. Subjective improvement was very characteristic. He was semiambulatory and 1 week after the first course there was a gradual appearance of well being, an increase in appetite, and attempts to get out of bed. Repeated radiologic examinations showed a regression in the lung lesions. He is still receiving a maintenance dose of NSC-140117 tablets 1½ years after the start of treatment. He resumed his work 3 months ago.

DISCUSSION

Until now, it has been well known that currently available chemotherapeutic drugs are unlikely to eliminate or cure advanced pulmonary cancer. Many drugs have been used with various results (6–10). The best results were reported from Japan with bleomycin (NSC-125066) (68% response) (11). Unfortunately, the experience by American investigators in 82 patients resulted in an 8% response rate only (10).

The good results we achieved with NSC-140117 in the management of malignant lymphoma and chronic myeloid leukemia (1,2) encouraged us to try it in solid tumors. In a pilot study of various solid tumors, a response was encountered in multiple myelomatosis and in bronchogenic carcinoma. Because of this we extended our studies to bronchogenic carcinoma using the present schedule. The results are encouraging. There has been a good response in nine of 15 patients (60%). Response was gradual and is still being maintained up to 1½ years from the start of treatment. The lack of drug toxicity encourages us to continue maintenance therapy. It also stimulates us to conduct a further study on bronchogenic carcinoma with even larger doses up to dose-limiting toxicity.

We can conclude that NSC-140117 has a role in the management of bronchogenic carcinoma.

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5-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) in the Treatment of Childhood Malignancy [1,2,3]

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5-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) was originally developed as a possible inhibitor of purine synthesis (1). Although its mustard structure suggests that its antitumor activity may be as an alkylating agent, Shealy et al (2) have presented evidence of antimetabolite activity through conversion to a diazeno component.

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[3] NSC-82196 was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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Animal tumor studies showed that the drug has a marked effect in the L1210 leukemia system (2) and the Walker 256 carcinosarcoma system (3). Phase I studies by the Southwest Oncology Group (4) with 5-day courses of the intravenous (iv) preparation showed a lack of prohibitive toxicity and antitumor effect at doses lower than 900 mg/m²/day. Phase II studies in adults with breast (5) and gastrointestinal (6) cancer failed to show favorable response rates. However, patients with malignant melanoma (7) showed at least a partial response in four of 20 trials. Previously reported clinical trials in children with acute leukemia have been limited to treatment with the oral form of NSC-82196 (Ɛ). Erratic absorption and rapid in vivo conversion of the drug to an inactive ionic transformation product were noted during these trials. No responses were noted in acute childhood leukemia treated with the oral form but responses were noted in three lymphoma patients treated at a dose of 0.8 g/m²/week.

A phase II study was designed by the Pediatric Division of the Southwest Oncology Group to test the efficacy of NSC-82196 in advanced acute leukemia and solid tumors of childhood. Because of the activity of the drug in L1210 leukemia, studies were also designed for children with advanced acute leukemia currently in remission who were resistant to known effective remission-maintenance agents. Data from children treated as part of the original phase I study are included as part of this report.

METHODS

Patient Selection

Children who were less than 15 years of age at the time of diagnosis of acute leukemia or metastatic solid tumor were eligible for the

TABLE 1.—Response and toxicity data for patients with solid tumors

Patient No.	Diagnosis	Prior chemotherapy*	Dose of NSC-82196 (mg/m²/day)	Response†	Toxicity‡
153–12	Neuroblastoma	V,C,AD	450	ID	L–1, T–0, N/V–2
			900	ID	L–2, T–3, N/V–2
381–1	Neuroblastoma	V,C,F,D	700	ID	L–NE, T–NE, N/V–1
			700	ID	L–NE, T–NE, N/V–1
381–16	Neuroblastoma	V,C,ADRI	600	ID	L–0, T–1, N/V–0
381–23	Neuroblastoma	C,V,P	800	PR	L–3, T–3, N/V–1
381–2	Neuroblastoma	V,C,ADRI,F	900	IMP	L–0, T–0, N/V–0
			1100	IMP	L–0, T–0, N/V–0
381–26	Neuroblastoma	C,V	900	ID	L–2, T–3, N/V–1
			900	ID	L–NE, T–NE, N/V–1
381–35	Neuroblastoma	C,V,C,AD,ADRI,F	900	ID	L–0, T–2, N/V–0
			900	ID	L–NE, T–NE, N/V–0
381–13	Neuroblastoma	P,V,AD,C,D	1100	ID	L–2, T–2, N/V–1
			1100	ID	L–1, T–2, N/V–1
381–21	Neuroblastoma	C,V,ADRI	1100	IMP	L–2, T–3, N/V–0
381–31	Neuroblastoma	C,V,ADRI	1100	ID	L–2, T–2, N/V–2
			1100	ID	L–1, T–0, N/V–1
381–24	Osteogenic sarcoma	C,V,ADRI	900	ID	L–0, T–0, N/V–2
381–25	Osteogenic sarcoma	C,V,ADRI,MPL	900	ID	L–0, T–0, N/V–1
381–37	Osteogenic sarcoma	F,ADRI,AP	900	ID	L–0, T–0, N/V–1
381–17	Osteogenic sarcoma	C,F,ADRI	900	ID	L–0, T–0, N/V–0
			1100	ID	L–0, T–0, N/V–0
381–28	Rhabdomyosarcoma	C,V,AD,ADRI,AP	800	ID	L–0, T–0, N/V–0
381–18	Rhabdomyosarcoma	V,C,AD,ADRI, MEC,F,PR	900	ID	L–1, T–2, N/V–1
381–34	Rhabdomyosarcoma	V,AD,C	900	ID	L–0, T–0, N/V–2
153–1	Ewing's sarcoma	V,C,AD,F,D,E	350	ID	L–0, T–0, N/V–2
			450	ID	L–1, T–0, N/V–2
381–27	Ewing's sarcoma	V,C,F,ADRI,AP	800	ID	L–0, T–0, N/V–0
381–36	Wilms' tumor	AD,V,ADRI,F,C	900	NE	L–0, T–0, N/V–2
153–2	Ependymoma	V	350	ID	L–0, T–0, N/V–3
381–10	Hepatoma	ADRI,A,F,C,MTX	600	NE	L–3, T–1, N/V–2
381–30	Mesothelioma	F,V,C	1100	ID	L–0, T–0, N/V–3

*A = L-asparaginase; AD = actinomycin D; ADRI = adriamycin; AP = 1-acetyl-2-picolinoylhydrazine; C = cyclophosphamide; CA = cytosine arabinoside; D = daunorubicin; E = emetine; F = 5-fluorouracil; MEC = mechlorethamine; MP = 6-mercaptopurine; MPL = melphalan; MTX = methotrexate; P = prednisone; PR = procarbazine; V = vincristine.

†NE = not evaluable (early death); ID = increasing disease; IMP = improvement; PR = partial response.

‡L = leukopenia; T = thrombocytopenia; N/V = nausea and vomiting. Nos. refer to degree of toxicity: 0 = none; 1 = mild; 2 = moderate; 3 = severe; NE = not evaluable.

study if they were resistant to standard therapy. Patients with acute leukemia in remission who were resistant to proven effective maintenance agents were eligible for the study of remission maintenance. Patients were excluded who had previously been treated with NSC-45388, the nitrosoureas (BCNU or CCNU), or streptozotocin; no patient with impaired renal or hepatic function was entered. Prior treatment is summarized in tables 1 and 2. Informed consent was obtained according to institutional guidelines.

Treatment Regimen

Patients with acute leukemia in relapse or solid tumors were treated with 350–1100 mg/m²/day iv for 5 days. Patients with impaired bone marrow reserve because of extensive prior radiotherapy were treated at the lower doses. Courses were to be repeated in 4–6 weeks.

Patients with acute leukemia in remission received 900 mg/m² in one dose every 2 weeks.

NSC-82196 was reconstituted with 0.01N HCl just prior to administration and the infusion concluded within 15 minutes after reconstitution.

Definition of Response

Complete response was defined as complete regression of all measurable lesions for patients with solid tumors or attainment of less than 5% bone marrow blast cells for patients with acute leukemia.

Partial response was defined as greater than 50% reduction in the product of the diameters of measurable lesions for solid tumors or less than 25% bone marrow blast cells for acute leukemia. Patients with less than 50% regression or subjective relief from bone pain were classified as improved.

Patients with acute leukemia in remission were classified as responders if they maintained less than 5% bone marrow blast cells for more than 56 days and if the length of their NSC-82196-maintained remission exceeded their most recent chemotherapy-maintained remission.

Length of response for solid tumor patients was defined as the time from the date of

TABLE 2.—Response and toxicity data for patients with acute lymphoblastic leukemia in relapse*

Patient No.	Dose of NSC-82196 (mg/m²/day)	Response	Prior chemotherapy	Toxicity†
153–8	350	ID	P,MP,D,V,MTX,A,CA,C	L–3, T–(3), N/V–2
153–27	800	ID	V,P,MP,MTX,D,C	L–3, T–0, N/V–3
	900	ID		L–3, T–3, N/V–3
153–30	900	ID	V,P,D,C,MP,A,F,CA,MTX	L–1, T–3, N/V–3
153–31	900	ID	V,P,MTX,MP,C,A,AD,F	L–2, T–3, N/V–3
381–2	900	ID	V,P,MTX,CA,C,A,ADRI	L–0, T–(3), N/V–1
	1100	ID		L–(3), T–(3), N/V–1
381–5	900	ID	C,A,V,P,MP,MTX,D,ADRI	L–(3), T–(3), N/V–3
381–6	900	ID	V,P,MP,C,A,MTX,D,AD	L–3, T–3, N/V–0
381–8	900	ID	V,P,MP,MTX,A,AD,ADRI,C	L–2, T–NE, N/V–1
381–12	900	ID	V,P,MTX,MP,A,C,ADRI	L–2, T–3, N/V–3
	1100	ID		L–3, T–3, N/V–3
381–11	1100	ID	V,P,MP,MTX,C,A,D	L–(3), T–(2), N/V–0
381–22	1100	ID	C,A,V,P,MTX,MP,ADRI	L–(3), T–(2), N/V–2

*See table 1 for definitions of prior chemotherapy, response, and toxicity.

†Parentheses indicate that leukopenia or thrombocytopenia was present before drug was given and cannot clearly be attributed to drug toxicity.

maximal improvement to the date of the first evidence of increasing disease. Length of remission for patients with acute leukemia in remission was defined as the time from the beginning of the NSC-82196 treatment (date of M_1 bone marrow status) to the date of M_3 bone marrow status (9). The length of response for patients with acute leukemia in relapse is not relevant since there were no responders.

Definition of Toxicity

Leukopenia was graded as follows for absolute neutrophil counts: mild = 1000–1499 cells/mm³; moderate = 500–999 cells/mm³; severe = less than 500 cells/mm³. Thrombocytopenia was graded as follows: mild = 75,000–99,999 cells/mm³; moderate = 25,000–74,000 cells/mm³; severe = less than 25,000 cells/mm³. Nausea and vomiting were graded as follows: mild = nausea only; moderate = antiemetics required to reduce vomiting to less than six times a day; severe = vomiting more often than six times a day despite antiemetics.

RESULTS

Response: Solid Tumors

Twenty-seven patients were entered in the study. Three patients were judged inevaluable for response because of major protocol violations: one patient was inevaluable for response because of intolerable toxicity (allergic reaction) and two patients registered never received therapy. The 23 evaluable patients received 32 courses of NSC-82196 (table 1).

One partial response and three improvements were seen in four patients with neuroblastoma. Despite these responses, these patients died from tumor within 2 weeks of their maximal response. The remainder of the solid tumor patients had increasing disease while receiving treatment.

Response: Acute Leukemia

Induction.—Thirteen patients with acute lymphoblastic leukemia in relapse were entered in the study. One patient was judged inevaluable because of intolerable toxicity (allergic reaction); one was inevaluable due to death during the first week of therapy. Eleven patients were evaluable for 14 courses of NSC-82196 (table 2). All patients with leukemia in relapse had increasing disease while receiving therapy. No patient with bone pain had relief from such pain.

Remission maintenance.—Five patients with acute lymphoblastic leukemia in remission were treated in the study (table 3). Two patients (Nos. 382–2 and 382–3) had remissions that were 14 and 32 days longer than their previous remissions. These prior remissions, however, were maintained with 5-fluorouracil as part of a phase II study. No remission exceeded 56 days, the anticipated maximal length of unmaintained remission.

Toxicity

Nausea and vomiting.—Nausea and vomiting were seen in 34 of 46 courses of NSC-82196. Two of 11 courses at doses between 350 and 900 mg/m²/dose for 5 days and seven of 31 courses at doses of 900 mg/m²/dose for 5 days were associated with severe nausea and vomiting. Lesser degrees of nausea and vomiting were slightly more prevalent at lower doses

TABLE 3.—Length of remission and toxicity data for patients with acute lymphoblastic leukemia in remission

Patient No.	Length of prior remission (days)	Length of current remission (days)	Toxicity*		
			Leukopenia	Thrombocytopenia	Nausea/ vomiting
382–1	70	21	0	0	1
382–2	17	49	0	0	1
382–3	14	28	1	0	0
382–4	28	20	(3)	(3)	2
382–5	42	28	0	0	0

*Toxicity rating: 0 = none; 1 = mild; 2 = moderate; 3 = severe. Parentheses indicate that leukopenia or thrombocytopenia was present before drug was given and cannot clearly be attributed to drug toxicity.

(table 2). Severe nausea and vomiting were not seen in the five patients receiving 900 mg/m² as a single dose every other week.

Leukopenia.—Severe leukopenia (absolute neutrophil count < 500 cells/mm³) was more prevalent at doses of 900 mg/m²/dose or greater and in patients with leukemia. In four of 14 trials in patients with leukemia, severe leukopenia was present before the drug was given and could not be clearly attributed to drug toxicity. The mean day of the leukocyte count nadir was similar in both leukemia groups.

Thrombocytopenia.—Severe thrombocytopenia (platelet count < 25,000 cells/mm³) was also more prevalent at doses of 900 mg/m²/dose or greater and in patients with leukemia. In six of 14 trials in patients with leukemia, moderate or severe thrombocytopenia was present before the drug was given. The mean day of the thrombocytopenia nadir for patients with leukemia was earlier (18.6 days) than for patients with solid tumors (28.2 days) (table 4).

Hypersensitivity.—Two patients had skin rash, generalized malaise, hypotension, and diarrhea. No further drug was given.

CONCLUSIONS

Of 32 courses of NSC-82196 in 23 children with solid tumors, the only good response was a short partial response in a child with neuroblastoma. Although three additional patients with neuroblastoma had either relief from bone pain or less than 50% regression, all "responders" were dead within 2 weeks.

None of 14 courses of NSC-82196 in 11 children with leukemia in relapse was associated with remission or relief from bone pain.

TABLE 4.—Nadirs for leukopenia and thrombocytopenia

	Leukopenia	Thrombocytopenia
Solid tumors		
No. of patients	10	11
Range of nadirs (days)	6–36	5–41
Mean nadir on Day—	18.4	28.2
Acute leukemia		
No. of patients	12	11
Range of nadirs (days)	4–42	3–29
Mean nadir on Day—	15.7	18.6

Two patients with advanced acute lymphoblastic leukemia in remission had a remission maintenance which was longer than their previous remission but was less than 2 months. This is not considered significantly longer than an unmaintained remission.

Nausea and vomiting were significant problems at all doses employed. Severe leukopenia and thrombocytopenia were dose related. They were also more prevalent in patients with greatly impaired bone marrow reserve (acute leukemia in relapse).

NSC-82196 is a highly insoluble and unstable compound that is rapidly converted to an inactive form in the light and at temperatures above freezing (11, 12). Rapid iv infusion within 15 minutes of reconstitution was used in an attempt to minimize photodecomposition during administration in a fashion similar to that previously reported (5–7) in phase II trials with the iv preparation. In none of these reports nor in our series was the infusate protected from light during the period of infusion, nor were pharmacologic studies performed to confirm the presence of active drug in the serum after infusion. However, since poor responses were obtained at doses with significant toxicity, further studies of this agent with more stringent environmental control and supportive pharmacologic studies do not appear warranted in childhood malignancies.

LIST OF COMPOUNDS

1-Acetyl-2-picolinoylhydrazine: NSC-68626; CAS reg. No. 17433-31-7
Actinomycin D: NSC-3053
Adriamycin: NSC-123127; CAS reg. No. 23214-92-8; doxorubicin
L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6
Emetine: NSC-33669; CAS reg. No. 316-42-7
5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8
Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; di-

ethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; PAM

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

NSC-45388: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; DTIC

NSC-82196: CAS reg. No. 5034-77-5; 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide; TIC mustard

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Streptozotocin: NSC-85998

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Combination Chemotherapy With Bleomycin (NSC-125066), Vincristine (NSC-67574), and Methotrexate (NSC-740) Plus Split-Course Radiotherapy in the Treatment of Non-Oat-Cell Bronchogenic Carcinoma[1,2]

Melvin L. Samuels, Howard T. Barkley, Jr., Paul Y. Holoye, Paul J. Rosenberg, and Terry L. Smith [3,4]

SUMMARY

Twenty-seven unselected patients with limited disease non-oat-cell bronchogenic carcinoma were treated with a chemotherapy-radiotherapy protocol which consisted of bleomycin, vincristine, and methotrexate followed by split-course radiation. There were 15 objective responders with a median survival time in excess of 70+ weeks in contrast to a median survival time of 26 weeks for nonresponders ($P<0.01$). Objective benefit was limited to the epidermoid carcinoma group since none of the adenocarcinoma group achieved a greater than 50% reduction in maximum tumor diameter. The median survival time for the entire group was 42 weeks in contrast to a recent split-course radiotherapy historical control group whose median survival time was 39 weeks. Toxic effects were predominantly gastrointestinal.

[Cancer Chemother Rep 59:377–383, 1975]

Radiation is the primary therapy for localized but clinically inoperable non-oat-cell bronchogenic carcinoma (so-called limited disease [1]). While such therapy is of unquestioned palliative value in many cases, a diversity of opinion exists concerning the benefits of radiotherapy when administered to asymptomatic patients. Early studies employing kilovoltage techniques have shown a distressingly low survival rate. The most frequently cited study by Roswit et al (2) revealed a median survival time of 4 months in 346 patients and 246 controls with a 1-year survival rate of 22% for the radiotherapy group versus 16% for the control group. Early supervoltage therapy reports did not appear to show further significant improvement either. The study of Boyd et al (3) gives a median survival time of 5 months and a 1-year survival rate of 25%. Krant et al (4) found no difference in kilovoltage versus supervoltage therapy as did Carr, Childs, and Lee (5). Hence, it was appropriate for investigators to turn to chemotherapy plus radiotherapy protocols. Studies have been reported which have combined various single agents with radiotherapy, such as mechlorethamine (4), vinblas-

[1] Received July 1, 1974; revised Sept 9, 1974; accepted Sept 25, 1974.

[2] Bleomycin was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text.

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tine (6), procarbazine (7), and hydroxyurea (8) with no significant improvement in survival having been seen. A multiple-drug program employing sequential two-drug combinations, primarily cyclophosphamide plus methotrexate and actinomycin D plus vincristine with split-course irradiation, has recently been reported by Hansen et al (9). The median survival time was 52 weeks for the responders and 18 weeks for the nonresponders. Although this difference was held to be statistically significant, either historical or prospective control data relative to split-course radiotherapy alone were lacking.

We have been interested in the combination of periwinkle alkaloids and bleomycin and have found clinical evidence to suggest their enhancement of effect upon stage III testicular neoplasia (10). Also, there are experimental observations which suggest a potentiation of the effect of bleomycin by the addition of vincristine.[5] Since methotrexate has also been demonstrated to possess activity against squamous bronchogenic carcinoma (11), a three-drug program combining bleomycin with vincristine and methotrexate followed by sequential split-course radiotherapy seemed logical for a pilot study.

MATERIALS AND METHODS

Twenty-seven unselected patients with bronchogenic carcinoma were entered into the study between August 1, 1971, and December 31, 1972. All patients were in the limited disease category and were ambulatory. They were treated on an outpatient basis except for a 79-year-old man. Patients were admitted to the study irrespective of symptom status. The details of the patient population are given in table 1. Bleomycin was given intramuscularly in a fixed dose of 15 units twice weekly for 3 weeks; vincristine was given intravenously in a fixed dose of 2.0 mg once weekly for 3 weeks; and methotrexate was given orally in a dose of 25–30 mg twice weekly for 3 weeks. After a 2-week rest period, split-course radiotherapy was started. A mid-plane dose of 3000 rads in ten fractions over a 2-week period was given for the first course. The patient was then re-evaluated after a 4-week rest period. If there was no evidence of distant metastases, a second course of 3000 rads was given over a period of 2 weeks (with the spinal cord being excluded for the final 1500 rads).

Chemotherapy was resumed after recovery from radiotherapy, usually within 2–4 weeks. The drug dosage, schedule of administration, and duration of therapy were identical to the initial course of treatment. Objective response was classified as (*a*) complete; (*b*) partial with at least a 50% decrease or more in the longest diameter; or (*c*) partial with less than a 50% decrease in the longest diameter. A single measurement was used since this was found to be more reproducible (12). Response was determined at the completion of the first course of chemotherapy and at the completion of radiotherapy. Further measurements were usually quite difficult because of fibrotic changes in juxtaposition to the tumor bed. Thus, the duration of objective response could not be accurately assessed. Complete blood counts were checked three times weekly. Blood gases and spirometry were done prior to chemotherapy and usually at completion of radiotherapy.

RESULTS

Objective response was noted in 15 of the 27 patients entered in the study. The data are summarized in tables 2 and 3. There were five complete responses, four partial responses of a greater than 50% reduction in maximum diameter of tumor, and six partial responses of a less than 50% reduction in maximum diameter of tumor. Seven of the responders (26%) showed response to the chemotherapy alone and in three of these patients this response was less than 50%. Although near complete responses were seen in two patients (one patient is shown in fig 1), there were no complete responses to chemotherapy. All of the responding patients showed a response to radiation. Figure 2 compares the survival curves of responders with nonresponders. The difference

[5] Schabel FM, Jr. Synergism and antagonism among antitumor agents. Presented at the 27th Annual Symposium in Fundamental Cancer Research (Pharmacological basis of cancer chemotherapy), M. D. Anderson Hospital and Tumor Institute, Houston, Tex, Feb 27–Mar 1, 1974.

TABLE 1.—Patient population*

Tumor histology	No. of patients	Mean age (yrs)	Sex (M/F)	Median duration of disease† (wks)	No. of patients who received previous thoracotomy
Squamous	21	59	19/2	6	9
Adenocarcinoma	6	50	4/2	2	0

*All but 1 treated as an outpatient.
†From diagnosis to treatment.

between these curves is statistically significant ($P<0.01$).

Four of the 15 responding patients and seven of the 12 nonresponding patients were unable to complete the second course of radiotherapy. This was due to new metastatic disease or pleural effusion in six patients, rapidly progressive disease with early death in one patient, bleomycin lung toxicity in one patient, severe radiation esophagitis in one patient, severe stomatitis in one patient, and an advanced age of 79 in one patient.

A comparison of responders and nonresponders is given in tables 4 and 5. General parameters such as pulmonary function status, percentage of weight loss, depression of serum albumin and hemoglobin levels, elevation of platelet counts, and elevation of serum lactic dehydrogenase levels were compared. Many patients with lung cancer enter therapy with substrate emphysema which can be an important factor in survival. However, in our study both the responders and nonresponders had the same mean and median PO_2 and forced vital capacity at 1 second (FVC_1) indicating that they are comparable in this respect. Of the remaining nonspecific parameters, the only significant difference appears to be in baseline platelet values. Of the seven patients with thrombocytosis, six were responders. The significance of this finding is unknown.

There were 17 deaths, nine of which were due to failure of local control of disease within the chest. Five of the nine patients had the full course of radiotherapy while four completed only 3000 rads given over 2 weeks. Four patients died as a result of brain metastases and one died from liver metastases. The remaining deaths were cardiovascular. Toxic effects were primarily gastrointestinal. Ten patients had severe stomatitis which usually occurred in the second week after the fourth dose of bleomycin. Often, the fifth or sixth dose of bleomycin had to be skipped. One patient had to be hospitalized for parenteral fluids. Although dysphagia from radiation esophagitis was seen to some degree in almost all patients, it was severe in only seven. Interstitial pneumonitis secondary to bleomycin occurred in one patient receiving bleomycin at 300 units. This was reversible. Leukopenia, unassociated with infection, occurred in five patients and leukopenia with thrombopenia occurred in one patient. There were three episodes of pneumonia, one during chemotherapy and two after radiotherapy. These patients recovered from these effects after hospitalization.

TABLE 2.—Response

Tumor histology	No. of patients	No. of patients— With complete regression	With partial regression*	Still alive	Mean survival of responders (wks)
Squamous	21	5	8	9	64
Adenocarcinoma	6	0	2	1	73,70

*Four squamous and 2 adenocarcinoma patients with <50% response.

TABLE 3.—Clinical data of patients with objective response

Tumor histology	Patient No.	Age (yrs)	Sex	Response	Survival (wks)	No. of chemotherapy courses	No. of radiotherapy fractions
Squamous	1	79	M	Complete	54+	1	1
Squamous	2	40	M	Complete	62+	4	1
Squamous	3	62	M	Complete	72+	2	2
Squamous	4	53	M	Complete	88+	2	1
Squamous	5	53	M	Complete	98+	1	2
Squamous	6	49	M	Partial >50%	52+	2	2
Squamous	7	56	M	Partial >50%	82+	4	2
Squamous	8	61	M	Partial >50%	20	1	1
Squamous	9	50	M	Partial >50%	70	2	2
Squamous	10	62	M	Partial <50%	67	3	2
Squamous	11	62	M	Partial <50%	81+	2	2
Squamous	12	75	M	Partial <50%	56+	1	2
Squamous	13	62	M	Partial <50%	38	1	2
Adenocarcinoma	14	65	M	Partial <50%	70	2	2
Adenocarcinoma	15	68	M	Partial <50%	73+	1	2

TABLE 4.—Comparison of responders and nonresponders

	No. of patients	Mean age (yrs)	Median*		No. of patients with adenocarcinoma	No. of patients completing radiotherapy†	Median survival in wks (range)
			PO_2 (mm Hg)	FVC_1			
Responders	15	60	72	50%	2	11	70‡ (20–98)
Nonresponders	12	55	71	55%	4	5	26 (6–48)

*As an index of the severity of baseline emphysema.
†Full split-course radiotherapy, 5500–6000 rads in 2 courses.
‡Projected.

TABLE 5.—Comparison of responders and nonresponders—baseline parameters

	No. of patients					
	Total	With weight loss (>5% of usual weight)	With low albumin (<3.5 g/100 ml)	With low hemoglobin (<12.0 g/100 ml)	With high platelets (>400,000 platelets/mm³)	With elevated LDH*
Responders	15	4	1	3	6	2
Nonresponders	12	5	3	4	1	3
Totals/%	27	9/33	4/15	7/25	7/25	5/18

*Only 1 nonresponder with high value of 477 IU/ml; remainder all <300 IU/ml.

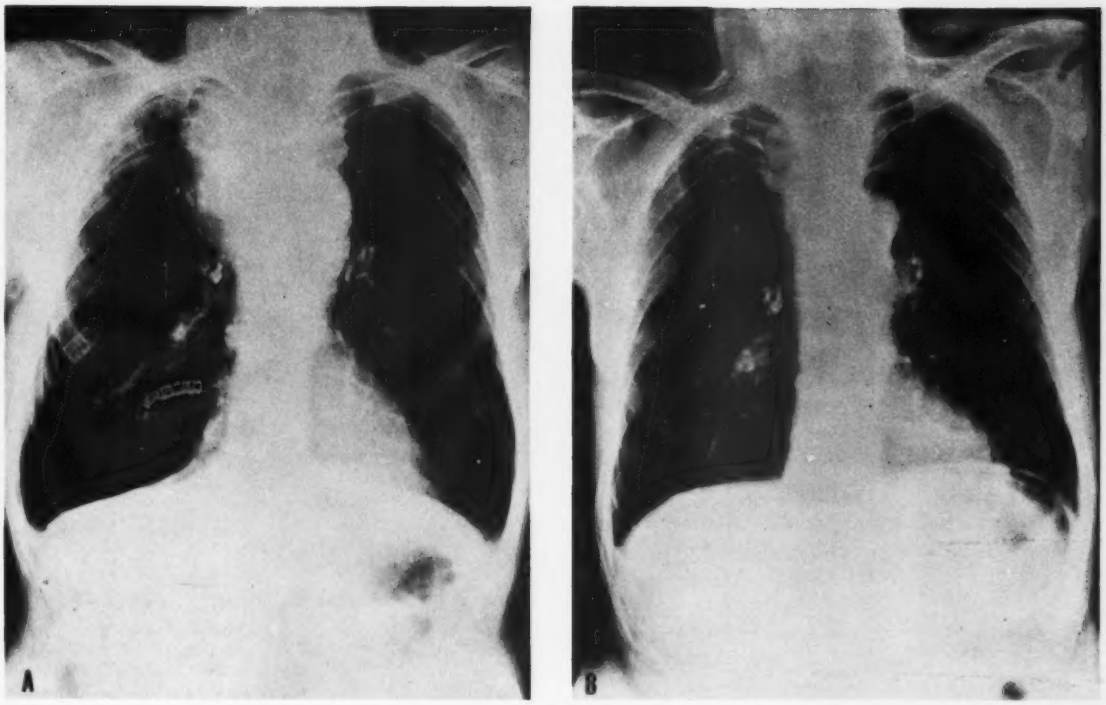

FIGURE 1.—(A) Chest roentgenogram of a 79-year-old white man with squamous bronchogenic carcinoma before treatment. (B) Chest roentgenogram after he had received the first course of chemotherapy prior to starting radiotherapy.

DISCUSSION

It is impressive that there was a 55% response rate with a median survival time in excess of 70+ weeks for responders in contrast to a 26-week survival time for nonresponders. This suggests that chemotherapy may be contributory. Further, the median survival time of the 21 responding and nonresponding patients with squamous histology is 52+ weeks which compares favorably with a Veterans' Administration placebo-treated squamous group with a survival time of 14–27 weeks (depending on performance status). Our patient population was ambulatory and most were considered in the best performance status category. Comparison with a similar Veterans' Administration group treated only with placebo shows an almost twofold increase in median survival time (13).

In an attempt to obtain a historical radiotherapy control, we have reviewed the records of 58 patients with non-oat-cell bronchogenic carcinoma who were treated from 1970 through 1972 with split-course radiotherapy only. Treatment was administered in the same manner as in our present study. The median survival time of this control group was 39 weeks (fig 3) as compared to 42 weeks for our 27 study patients ($P = 0.49$). Thus, we are unable to demonstrate a statistically significant improvement in overall survival time by the addition of chemotherapy to split-course radiotherapy. Figure 4 contrasts a semilog plot of the cyclophosphamide, methotrexate, split-course radiotherapy study reported by Hansen et al (9) with our combined therapy and control patients. There is no significant difference between the two chemotherapy-radiotherapy groups (whose curves plot approximately as straight lines),

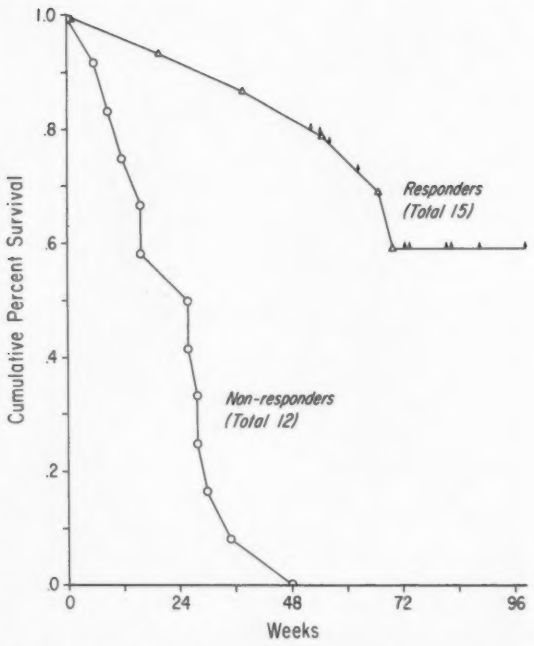

FIGURE 2.—Comparison of the survival curves of responders (△) and nonresponders (○). The difference between the curves is statistically significant ($P < 0.01$, modified Wilcoxon test).

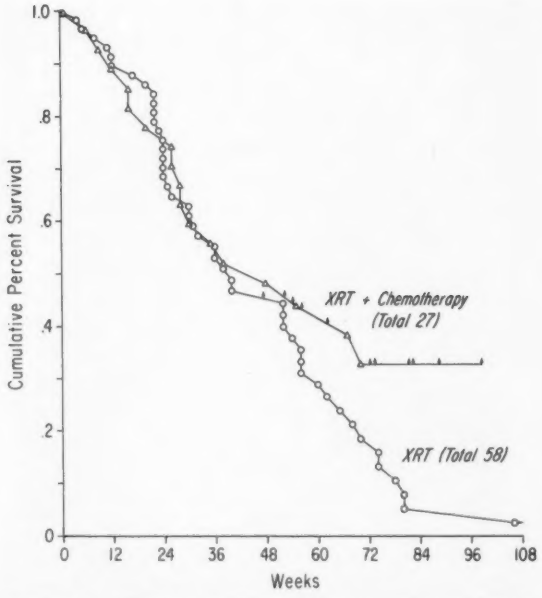

FIGURE 3.—Survival curves of the chemotherapy-radiotherapy group (△) vs a recent historical split-course megavoltage control group (○) (M. D. Anderson Hospital). There is no statistically significant difference ($P = 0.49$, modified Wilcoxon test).

indicating a constant death rate. The death rate was also fairly constant through the first year for the radiotherapy control patients but dropped rapidly thereafter. This suggests that chemotherapy may make a contribution that becomes apparent on long-term followup.

While the survival rates of the two chemotherapy-radiotherapy studies are similar, it appears that the cyclophosphamide-methotrexate protocol of Hansen et al (9) is superior for adenocarcinoma. Five of the six evaluable adenocarcinoma patients studied in this program achieved remission for a median survival time of 510 days while only two of the six adenocarcinoma patients in our protocol achieved less than a 50% response with a median survival of only 196 days for the group.

Radiotherapy remains the cornerstone of present-day management of limited disease non-oat-cell bronchogenic carcinoma. We feel radiotherapy is best administered by the split-course technique. The study of Rissanen (14) suggests that local control in bronchogenic squamous carcinoma is better with split-course rather than continuous therapy with a tumor dose above 4800 rads. Another major advantage of split-course therapy is the added time the clinician has for observing the development of metastases. Six of our patients (22%) had clinically evident metastases prior to the projected second course. Thus, these patients were saved unnecessary irradiation and valuable machine time was conserved.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053

Bleomycin: NSC-125066

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan

Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; HN2; nitrogen mustard

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-

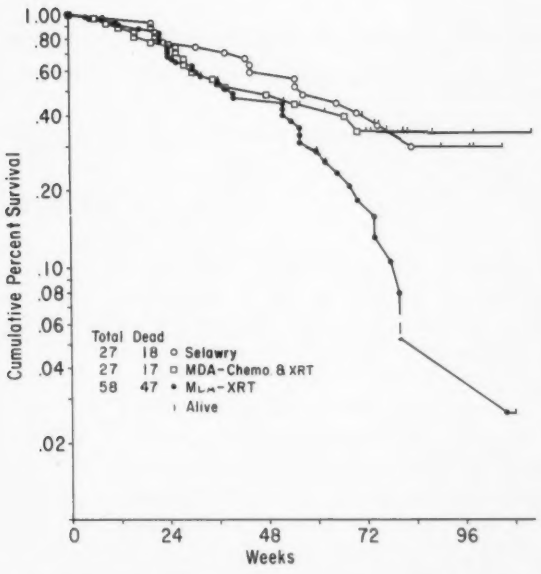

FIGURE 4.—Semilog plot of the survival of the Hansen-Selawry chemotherapy-radiotherapy group (○) (*see* text and ref 9) vs a combined therapy group (□) and a control group (●) from the M. D. Anderson Hospital. Survival in the Hansen-Selawry group was not significantly longer than the M. D. Anderson combined treatment group ($P = 0.41$, modified Wilcoxon test).

toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2, leurocristine, sulfate (1:1)

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Acute Monocytic Leukemia in Children and its Response to Vinblastine (NSC-49842)[1,2,3]

Clementina F. Geiser and Jack W. Mitus[4,5]

SUMMARY

Seventeen children with acute monocytic leukemia were treated with (*a*) vinblastine (with or without prednisone), 0.2 mg/kg of body weight twice a week (13 patients); (*b*) 6-mercaptopurine plus prednisone (three patients); or (*c*) cyclophosphamide plus prednisone (one patient). Four patients died within the 1st week, before having an adequate chemotherapeutic trial, and a fifth patient died of sepsis at 12 days. Five patients achieved complete remissions and two achieved good partial remissions. The total remission rate for the 17 patients in this study was 41%. For the 13 patients who survived more than 1 week, the remission rate was 53.8%. Remissions occurred with vinblastine in all but one patient. Of the ten patients who received more than one dose of vinblastine, 60% achieved a remission, indicating that vinblastine is an active agent in this rare form of leukemia.

[Cancer Chemother Rep 59:385–388, 1975]

This report summarizes the treatment of acute monocytic leukemia (AMoL) at the Sidney Farber Cancer Center (formerly the Children's Cancer Research Foundation) from 1960 to 1972. AMoL in this series of patients corresponds to the pure, or Schilling type, monocytic leukemia of the early nomenclature (1), as distinct from the acute monomyelogenous leukemia (AMML), Naegeli type, from which it differs in frequency and morphology.

AMoL is very rare. Five cases of AMoL versus 66 of AMML were found among 627 adults with leukemia (2). In 679 pediatric cases of leukemia, there was one case of AMoL versus 40 of AMML (3), and among 1263 leukemic children treated at this center between 1947 and 1965, there were 27 cases of AMoL (2.1%) versus 66 of AMML (5.1%) (4). Ten cases of AMoL were reported recently in 130 pediatric and adult cases of leukemia (5). In AMoL the marrow is replaced by monoblasts: immature cells with round or folded nucleus, fine chromatin network, usually one large nucleolus, and abundant cytoplasm with a gray "ground glass" appearance due to fine acidophilic and basophilic granulations. The term monohistiocytic or histiocytic leukemia is used when the blast cells have prominent pseudopods and resemble histiocytes. Monoblasts are peroxidase negative, show a negative reaction for napthol AS-D chloroacetate esterase (6), and have a

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[2] Supported by Public Health Service grant CA-06516 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are included in the List of Compounds at the end of the text. Vinblastine was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

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characteristic sodium fluoride inhibition of their highly active nonspecific esterases (7,8).

In AMML, the marrow shows, by contrast, distinct myeloid hyperplasia and an increased percentage of peroxidase-positive myeloblasts, associated with a variable percentage of cells of the monocytic series with some immature monoblasts.

All patients with AMoL and most patients with AMML have an abnormally high level of muramidase (lysozyme) in the serum and excrete it in the urine in large amounts (9,10).

CLINICAL MATERIAL

AMoL was diagnosed by marrow examination in 17 patients (ten girls and seven boys; ages ranged from 6 months to 15 years). In eight cases the blast cells suggested monohistiocytic leukemia. Esterase studies were not done. Serum muramidase was 20 and 100 μg/ml, respectively, in two patients (normal, $<10\mu$g/ml) and dropped to 4.6 μg/ml during remission in the latter case. Marrow karyotype was normal in the three patients tested. At diagnosis, two patients had skin nodules which on biopsy revealed monocytic leukemia of the dermis and subcutaneous tissues. The liver, spleen, and lymph nodes were not enlarged in 40% of the patients, and were moderately so in the others. Kidneys were palpable in one patient. Gum hypertrophy was present in three patients, subperiosteal infiltrations were seen on X-ray in two patients, bilateral nerve palsy was present in one patient, and hepatitis with hypoproteinemic edema was present in one other patient. The initial leukocyte count ranged from 1200 to 328,000 cells/mm^3, the blast cell count ranged from 2 to 220,000 cells/mm^3, and the platelet count ranged from 9000 to 220,000 platelets/mm^3. The three patients who died early due to cerebral hemorrhage had leukocytosis ($>$ 200,000 cells/mm^3 with $>$90% blast cells).

CHEMOTHERAPY AND RESULTS

After diagnosis, one patient received cyclophosphamide plus prednisone without response. Three patients received 6-mercaptopurine (6-MP) plus prednisone: of these patients, one achieved a complete marrow remission which was maintained for 3 months on 6-MP, one did not respond, and the third did not have an adequate trial due to a fatal cerebral hemorrhage in the 1st week.

Of the remaining 13 patients, ten received vinblastine, 0.2 mg/kg of body weight twice a week, and three received the same dose once a week. There was no obvious toxicity during the vinblastine therapy. Seven patients received concomitant prednisone, 1 mg/kg/day. Three of the 13 patients died within 1 week from diagnosis: two with cerebral hemorrhage and one with jaundice, having received only one dose of vinblastine. A fourth patient died of sepsis after 12 days, having received prednisone and two doses of vinblastine. Therefore, at least three of the four patients who died early did not have an adequate trial with vinblastine.

Of the ten patients treated with vinblastine who survived more than 1 week, four achieved complete marrow remissions ($<$ 5% blast cells) and two achieved good partial remissions (5%–10% blast cells), bringing the total number of vinblastine responders to six. In addition, another patient had clinical improvement with a reduction of blast cells in the marrow from 86% to 34% (table 1). Marrow remission was documented after 4 weeks of therapy in two cases, 5 weeks in one case, and 7 weeks in one other case. Chemotherapy was discontinued at the time of remission and relapse occurred between 2 and 11 weeks later. Attempts to obtain a second remission failed in all but one patient who obtained another short remission with 6-MP plus prednisone.

DISCUSSION

Although it is commonly thought that AMoL responds poorly to chemotherapy, the rarity of this form of leukemia makes it difficult to evaluate its response to therapy. In one series of 32 children with acute leukemia, five were listed as having monocytic leukemia (a surprisingly high percentage), and three of them achieved remissions with vincristine (11). In another series of 15 pediatric and adult patients with AMoL receiving combination chemotherapy with prednisone, methotrexate, vincristine, and methyl-GAG, only two (14%) achieved complete remissions while three had partial remissions, with a total of 33% responders (12). Among five adults with AMoL treated with

TABLE 1.—Response to chemotherapy in 17 children with AMoL

Response*	Total No. of patients	No. of patients receiving— Vinblastine plus prednisone	Vinblastine alone	6-MP plus prednisone	Cyclophosphamide plus prednisone
Complete remission	5	2	2	1	—
Good partial remission	2	2	—	—	—
Improved	1	—	1	—	—
No response	5	2	1	1	1
Inadequate trial	4	1	2	1	—
Total	17	7	6	3	1

*Complete remission = <5% blast cells in the marrow; good partial remission = 5%–10% blast cells in the marrow; improved = blast cells in the marrow decreased from 86% to 34%.

L-asparaginase and daunorubicin, only one achieved a remission, however, without remission of his skin lesions (13). Recently, remissions were reported in six of ten pediatric and adult patients with AMoL, utilizing various regimens of combination chemotherapy which included prednisone, vincristine, cytosine arabinoside, and anthracyclines (5).

In our group of 17 patients, five achieved complete remissions and two achieved good partial remissions, with a total remission rate of 41%. However, for the 13 patients who survived more than 1 week, the remission rate was 53.8%. In six patients remission was achieved with vinblastine, with or without prednisone, and in one patient, remission was achieved with 6-MP plus prednisone.

Of the ten patients who received more than one dose of vinblastine, 60% achieved a remission, indicating that vinblastine has a definite effect on the blast cells of AMoL. The effectiveness of vinblastine in the treatment of AMoL, as contrasted to its poor performance in the other forms of leukemia, was first noted during the early vinblastine trials in 1960 when the first patient in this series obtained a remission. This is not surprising, in view of the effectiveness of vinblastine in histiocytosis and Hodgkin's disease which contain some cell populations related to the monohistiocytic series. Although the remission rate with vinblastine has been unusually high in our patients, the length of remission has been quite disappointing. This is probably due to the premature discontinuation of therapy in early remission, and the absence of an intensive maintenance program with combination chemotherapy.

The experience with this group of patients suggests that vinblastine is of value in the treatment of AMoL and that it may be considered in the formulation of chemotherapeutic protocols for this rare form of leukemia.

LIST OF COMPOUNDS

L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; arabinosyl cytosine
Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6
6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate; 6-MP
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-
Methyl-GAG: NSC-32946; CAS reg. No. 24968-67-0; guanidine, 1,1'-[(methylethanediylidene)dinitrilo]di-, dihydrochloride, monohydrate
Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate
Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Phase I Evaluation of Cyclocytidine (NSC-145668)[1,2,3]

Jacob J. Lokich,[4] Prem L. Chawla,[5] Norman Jaffe,[4] and Emil Frei III [4,6]

SUMMARY

Cyclocytidine is a repository form of cytosine arabinoside and has a prolonged plasma half-life (8 hours) compared to cytosine arabinoside (12 minutes). A phase I trial was undertaken to establish the maximum tolerated dose. Nineteen patients were entered into the study (ten children, nine adults) and the dose which achieved consistent myelosuppression was established in five escalation increments as 600 mg/m² for 10 days. This dose is consistent with previously reported data on the basic pharmacology of cyclocytidine and with optimum dose data in animal tumor systems. Limiting toxicity, however, was related to the induction of postural hypotension which is not dose related. Recommendation to proceed with phase II studies of cyclocytidine must await clarification of the mechanism and prevention of the postural hypotension.

[Cancer Chemother Rep 59:389–393, 1975]

Cytosine arabinoside [7] is a pyrimidine nucleoside analog that has important antitumor activity in experimental tumor systems in animals and in hematologic tumors in man (1,2). A major feature of the antitumor effect relates to the schedule of administration (2). Continuous intravenous (iv) infusion in man and frequent parenteral administration of cytosine arabinoside in experimental tumors produces a superior therapeutic index (3). Clinical pharmacology studies indicate that this drug is rapidly degraded by deaminases and has a plasma half-life of only 12 minutes. The inconvenience of continuous iv therapy with cytosine arabinoside has led to attempts to develop analogs which would provide longer plasma levels.

Cyclocytidine is the anhydro derivative of cytosine arabinoside. The comparative structures are illustrated in figure 1. The basic chemical alteration is the formation of an oxygen bridge at carbon 2. Cyclocytidine is not a substrate for deoxycytidine deaminase, which catabolizes 80% of cytosine arabinoside in man, but undergoes slow hydrolysis to cytosine arabinoside. This results in a plasma half-life of the active product (cytosine arabinoside) of 8 hours (4). In animal tumor systems, Hoshi et al and Venditti et al have established that cyclo-

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[2] Supported by Public Health Service grant CA-06516 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Cyclocytidine: CAS reg. No. 10212-25-6; 6*H*-furo-[2′,3′:4,5]oxazolo-[3,2-*a*]pyrimidine-2-methanol, 2,3,3*a*,-9*a*-tetrahydro-3-hydroxy-6-imino-, monohydrochloride; 0³,2′-cyclocytidine, monohydrochloride; cyclo-C. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

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[7] Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C.

cytidine is more active than cytosine arabinoside in L1210 leukemia with comparable schedules. In addition, cyclocytidine is active against Adenocarcinoma 755, ascites Sarcoma 180, Ehrlich ascites sarcoma, and C-1498 leukemia (5,6).

A clinical phase I evaluation was initiated to determine the qualitative and quantitative toxicity of cyclocytidine and to establish the maximum tolerated dose.

MATERIAL AND METHODS

Nineteen patients with advanced neoplastic disease were entered into the phase I study. All patients had histologic confirmation of malignancy and fulfilled the eligibility criteria for phase I study. These criteria included normal renal and hepatic function documented by biochemical tests, normal hematologic parameters, an expected survival time of at least 2 months, and the absence of previous radiotherapy or chemotherapy for a minimum period of 4 weeks. For patients with acute leukemia, hematologic parameters were not restrictive nor did prior exposure to cytosine arabinoside preclude cyclocytidine therapy. All of our patients had symptomatic disease as well as objective measurable tumor and they had become resistant to established therapy (15 patients) or were considered candidates for a primary phase I trial (four patients). Fifteen of 19 patients had had prior therapy. Eleven patients had had prior chemotherapy (including seven with previous cytosine arabinoside therapy and four had had prior radiotherapy (three of the latter involved major areas of marrow reserve). The categories of neoplastic disease included 12 patients with solid tumors (breast two, colon three, and one each with pancreas, ovary, lung, lymphoma, Ewing's, fibrosarcoma, and Wilms' tumor) and seven patients with acute leukemia (table 1). Ten of the 19 patients studied were less than 10 years of age.

The initial treatment plan was to start patients at two dose schedules: (*a*) daily for 5 days every 2 weeks, and (*b*) daily for 10 days every 19 days. Thus a minimum of 9 days was allowed between courses. Dose escalation was accomplished in 50% increments for each cycle unless toxicity occurred with the prior course in the same or other patients (in which case appropriate modifications were made). Three patients were entered at or achieved a given dose level before the dose was increased. The number of trials administered at each dose level and schedule is presented in table 2. The starting dose for the phase I study was calculated on the basis of large animal toxicology studies in which the toxic dose (low) for monkeys and dogs was 240 and 400 mg/m², respectively.

Patients were monitored with complete blood counts including platelet counts and differential twice weekly and liver and renal function tests weekly. Tumor measurements by radiologic and

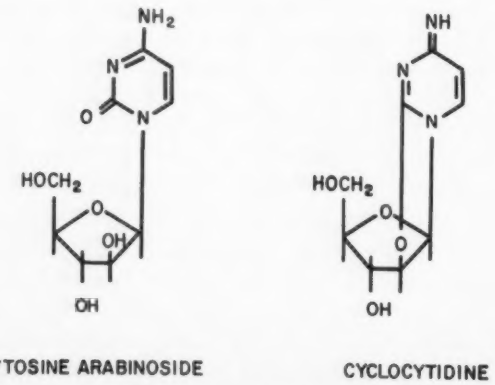

FIGURE 1.—Comparative structures of cytosine arabinoside and cyclocytidine.

TABLE 1.—Clinical features of 19 patients entered into the cyclocytidine phase I study

Primary disease	No. of patients
Acute leukemia	
Lymphocytic	4
Myelogenous	3
Solid tumor	
Sarcoma	2
Epithelial	8
Lymphoma	1
Wilms'	1
Total	19
Prior therapy	
Chemotherapy	11
Radiotherapy	4
Total	15

TABLE 2.—Cyclocytidine phase I study: total No. of courses administered by dose and schedule

Schedule	No. of patients	No. of courses	Dose level (mg/m²/day) 100	150	225	300	500	600	1000
5-day	10	18	3	2	7	1	4	1	0
10-day	9	13	2	1	1	3	0	5	1*
Totals	19	31	5	3	8	4	4	6	1

*Patient received 1 g/day for 23 days.

physical examinations were performed at least monthly. Patients were removed from study for unacceptable toxicity or for the development of rapidly progressive disease after a minimum of two courses of therapy.

Cyclocytidine, which is stable without refrigeration, was provided in 100-mg vials as a lyophilized powder. The drug was reconstituted with 10 cc of water for injection and administered within 1 hour of preparation as a rapid iv injection on each of 5 or 10 consecutive days.

RESULTS

The initial dose regimen of 10 mg/m² of cyclocytidine given on a 5- and 10-day schedule was escalated according to protocol criteria to a 600-mg/m² dose. One patient was treated with 1000 mg/m² for 23 consecutive days (*see* table 2). Two basic patterns of toxicity secondary to cyclocytidine were observed. Constitutional symptomatology included gastrointestinal side effects with nausea (with and without emesis) and was observed in seven of 19 patients (30%); "jaw" pain was observed in eight of 19 patients (42%); and postural hypotension was observed in seven of 19 patients (30%) (table 3). Postural hypotension occurred only in adults and, therefore, was actually observed in 76% of the susceptible population. None of these side effects were dose related. Jaw pain developed shortly after the injection and lasted for approximately 7 hours. The pain was typically mild and did not require analgesics. Precise localization was difficult but occasionally sialorrhea was an associated phenomenon although there was no parotid tenderness and serum amylase levels were normal.

TABLE 3.—Frequency of constitutional toxicity with cyclocytidine

Toxic effect	No. of patients with effect/ No. treated
Nausea and/or vomiting	7/19 (30%)
Postural hypotension	7/19 (30%)*
Jaw pain	8/19 (42%)

*7/9 adults (76%).

Postural hypotension was a unique side effect of cyclocytidine which occurred at doses as low as 150 mg/m². The hypotension occurred approximately 30–60 minutes after injection and persisted for a period of 6–8 hours. Clinically, these episodes occurred with normal reflex tachycardia and responses to Valsalva's maneuver and were accompanied by symptoms of light-headedness. Patients were evaluated with regard to other etiologies of postural hypotension such as ancillary drug use, dehydration, adrenal insufficiency, etc, and no other causes could be established.

In general, these constitutional side effects were not dose or schedule related. The postural hypotensive effect, in particular, was consistent in severity and did not change in duration or quality of incapacity with successive courses given at higher doses to the same patients.

The second type of toxic effect of cyclocytidine was myelosuppression. This effect was dose related and the incidence of leukopenia and thrombocytopenia related to dose is detailed in table 4. Two patients had mild thrombocytopenia of 56,000 and 71,000 platelets/mm³ at low doses (150 and 300 mg/m², respectively) of cyclocytidine. Both patients had had previous chemotherapy with nitrosoureas. There were

TABLE 4.—Incidence of thrombocytopenia and leukopenia according to dose escalation

Dose (mg/m^2)	Schedule (days)	No. of trials	Incidence of— Leukopenia (<2500 cells/mm^3)*	Thrombocytopenia ($<10^5$ cells/mm^3)*
100	× 5	3	0	0
	× 10	2	0	0
150	× 5	2	0	1 (56,000)
	× 10	1	0	0
225	× 5	7	0	0
	× 10	1	0	0
300	× 5	1	0	1 (71,000)
	× 10	3	0	0
500	× 5	4	0	0
	× 10	0	0	0
600	× 5	1	0	0
	× 10	5	5 (2000)	5 (80,000)

*Nos. in parentheses = median level (cells/mm^3).

no other episodes of myelosuppression observed until the 600-mg/m^2 dose level was given. A single patient was entered on the 5-day schedule and 600-mg/m^2 dose and no myelosuppression was observed. However, in five trials with three patients on the 10-day schedule, moderate myelosuppression occurred with each course. Thrombocytopenia to a median level of 80,000 platelets/mm^3 and leukopenia to a median white blood cell count of 2000 cells/mm^3 were observed. There were no concomitant episodes of bleeding or infection as a consequence of myelosuppression. A child with acute myelogenous leukemia was treated with 1 g/m^2 for an extended period (23 days). This patient was markedly granulocytopenic and thrombocytopenic at the start of therapy and his condition did not change.

There were no significant objective responses to therapy. One patient with acute myelogenous leukemia demonstrated a decrease in the marrow blast cell count from 20% to 9% and, simultaneously, the peripheral blood counts returned towards normal. However, this circumstance lasted for only 7 days and was followed by relapse and progressive leukemia. Of the total group of patients studied, 16 of 19 have died with a median survival time of 2 months for the children and 5 months for the adults.

DISCUSSION

Cyclocytidine is a repository form of cytosine arabinoside and on the basis of clinical pharmacology studies and experimental antitumor activity it is superior to cytosine arabinoside. This phase I study was designed to establish the maximum tolerated dose and schedule of cyclocytidine and the qualitative and quantitative pattern of toxicity. Five dose increments were required to achieve consistent and moderate myelosuppression which was anticipated to represent the potential dose-limiting effect of the drug. Marrow suppression as manifested by thrombocytopenia and leukopenia was consistently observed at a dose and schedule of 600 mg/m^2 for 10 days. This dose level is consistent with the clinical pharmacology of cyclocytidine and the cyclocytidine-cytosine arabinoside comparative studies in L1210 leukemia. In the latter studies, cyclocytidine has an optimum dose level ratio relative to cytosine arabinoside of 5 or 10:1.

The optimal tolerable dose in clinical studies for cytosine arabinoside administered directly as the parent compound is 100–150 mg/m^2/day for 5 days. The optimal tolerable dose of cyclocytidine (with regard to comparable myelosuppression) is 600 mg/m^2 for 10 days which is 8–12 times the optimal dose of cytosine arabi-

noside. However, since only 15% of cyclocytidine is converted to cytosine arabinoside, these maximum doses for cytosine arabinoside and cyclocytidine are approximately equal.

However, the limiting toxic effect of cyclocytidine is related to the induction of postural hypotension and not myelosuppression. The mechanism of this toxic effect is unclear. Clinical studies of reflex control mechanisms indicate that they are essentially normal. The means by which cyclocytidine produces postural hypotension may be related to depletion of peripheral neuro-transmitter substances or competitive blocking of vascular receptor sites for vaso-constrictor substances.

The postural hypotensive effect of cyclocytidine represents the basic limiting toxic effect and develops at doses which are lower than those required to produce consistent myelosuppression. Furthermore, hypotension is a severe and profound side effect necessitating recumbency and constant monitoring. As such, this side effect obviates the potential advantage of cyclocytidine as a repository drug. Pharmacologic interruption of the hypotensive effect may be possible utilizing sympathomimetic or parasympatholytic agents and is presently being investigated.

Although not a specific objective of this phase I study, the antitumor effect of cyclocytidine was evaluated. Objective therapeutic responses were not observed in the study group although some patients experienced subjective relief of nonspecific symptoms.

In conclusion, cyclocytidine is a drug with major advantages over cytosine arabinoside on the basis of antitumor effect in animal systems and pharmacologic characteristics in man. The induction of acute postural hypotension in adult patients is a unique effect of cyclocytidine, and studies are presently in progress to attempt to pharmacologically interrupt or prevent this side effect. The transition of cyclocytidine to phase II studies should await the resolution of the etiology and control of this unusual side effect. However, postural hypotension is not observed in children and there are major theoretic advantages of cyclocytidine over cytosine arabinoside. Phase II studies for pediatric neoplastic diseases should commence with an initial dose of not less than 600 mg/m^2 for 10 days.

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Evaluation of Daunorubicin (NSC-82151) and Methotrexate (NSC-740) in Combination as a Remission Maintenance Regimen in the Treatment of Acute Leukemia[1,2,3]

G. B. Humphrey, D. J. Fernbach, A. A. Razek, W. J. Stuckey, D. Komp, and S. George[4,5]

SUMMARY

The addition of a single monthly dose of daunorubicin (25 mg/m² given intravenously) to the maximum tolerated dose of methotrexate (25 mg/m²/dose given orally twice weekly) was evaluated as a maintenance regimen in 70 children with acute lymphoblastic leukemia. Seven patients who were nonevaluable were excluded from the study; 31 patients received methotrexate alone and 32 patients received a combination of methotrexate and daunorubicin. There was no significant difference between the distribution of remission times for the two maintenance regimens (medians of 147 and 162 days, respectively). For patients with only one previous remission, the median length of remission in this study was significantly shorter than that of the original remission time. Despite the known myelosuppressive effects of both agents, daunorubicin did not cause additional toxicity nor did it interfere with the scheduled methotrexate therapy. Although the addition of daunorubicin to methotrexate does not appear to be beneficial in the treatment of acute leukemia, this combination could be evaluated in other cancers in which both agents are known to be effective.

[Cancer Chemother Rep 59:395–399, 1975]

Methotrexate is an effective remission maintenance agent in the treatment of acute lymphoblastic leukemia (ALL) in children. The duration of remission with intermittent (twice weekly) therapy has been demonstrated to be longer than that obtained with a daily treatment regimen (1). Daunorubicin is an effective agent in the treatment of acute myelogenous leukemia (AML) in children (2) and, although effective in ALL, the results of clinical trials have been variable (2–7).

In 1971, several member institutions of the

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[2] Supported by Public Health Service grants CA-11233, CA-03161, CA-05587, CA-03389, CA-08223, CA-12014, CA-04919, and CA-12644 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Daunorubicin: CAS reg. No. 23541-50-6; daunomycin; rubidomycin (NSC-83142).

Methotrexate: CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]-benzoyl]-; MTX.

Both drugs were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[4] This study was done by the Southwest Oncology Group. The following members participated in the study: *G. B. Humphrey* (University of Oklahoma School of Medicine, Oklahoma City); *D. J. Fernbach* (Baylor College of Medicine, Houston, Tex); *A. A. Razek* (Washington University School of Medicine, St. Louis, Mo); *W. J. Stuckey* (Tulane University School of Medicine, New Orleans, La); *D. Komp* (University of Virginia School of Medicine, Charlottesville); and *S. George* (University of Texas M. D. Anderson Hospital and Tumor Institute, Houston).

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Pediatric Division of the Southwest Oncology Group conducted a phase I study to evaluate the toxicity of a single monthly dose of daunorubicin added to the maximum tolerated biweekly dose (25 mg/m²) of methotrexate given orally (8). Seventeen patients who were entered into this study had previously received two or more courses of chemotherapy (vincristine, prednisone, 6-mercaptopurine, etc) [6] but had not received methotrexate or daunorubicin. Methotrexate was administered at a dose level of 25 mg/m² twice weekly. In addition, three different doses of daunorubicin, given at 28-day intervals by direct intravenous (iv) injection, were evaluated: 12.5, 25, and 50 mg/m².

The 25-mg/m² dose schedule was well tolerated. An evaluation of toxic effects in these patients evidenced very few problems, although therapy was occasionally interrupted or delayed because of myelosuppression. No increased gastrointestinal toxicity and no cardiac toxicity was noted in those children receiving accumulative doses ranging up to 500 mg/m². Because the length of remission was impressively prolonged in several patients, a phase III (randomized) study was undertaken to compare a standard methotrexate maintenance regimen with the same regimen plus periodic reinforcement with daunorubicin with respect to duration of remission. This paper reports the results of that study conducted by the Southwest Oncology Group.

MATERIALS AND METHODS

Seventy children who had had no prior exposure to methotrexate or daunorubicin, were less than 15 years of age at the time of the initial diagnosis of ALL, and had previously achieved a remission with vincristine and prednisone were registered in this study. Thus, all patients had relapsed at least once. Induction therapy was accomplished with a combination of vincristine, prednisone, and L-asparaginase.[7]

[6] Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); VCR.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate.

[7] L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3.

Patients who had achieved a complete remission were registered and randomly assigned to one of two regimens: (*a*) methotrexate, 25 mg/m²/dose twice weekly, or (*b*) methotrexate, 25 mg/m²/dose twice weekly plus a monthly iv infusion of daunorubicin, 25 mg/m². Daunorubicin therapy was limited to a cumulative dose of 500 mg/m² (20 doses). Patients were taken off the study when the bone marrow specimen contained more than 25% blast cells. The occurrence of meningeal leukemia did not terminate treatment, although occasional dose deletions or dose reductions of methotrexate were made during intrathecal methotrexate therapy. Figure 1 gives the schema outlining the treatment schedules.

RESULTS

Of the 70 registered patients, there were seven patients who were not evaluable for this analysis and four other patients who were only partially evaluable for the analysis. The four partially evaluable patients were patients who had experienced a major protocol violation at some time (such as omission of daunorubicin as scheduled) but who were fully evaluable until that time. Thus, there is incomplete or "censored" information on the length of remission for these patients. The reasons for declaring the seven patients nonevaluable and, hence, omitting them entirely from the analysis are given in table 1.

The distribution of remission times for the two maintenance regimens is given in figure 2. The median duration of remission was 147 days for methotrexate alone (31 patients) and 162 days for methotrexate/daunorubicin (32 patients). As is evident from the figure, there is no statistically significant difference between the two distributions of remission times (generalized Wilcoxon test, two-tailed, $P = 0.70$).

It is also of interest that for patients with only one previous remission on either treatment, the median length of remission in this study was significantly shorter than that of the previous remission (signed rank test, $P < 0.05$). For the methotrexate-alone regimen the median length of original remission was 255 days (19 patients) and for the methotrexate/

TABLE 1.—Case material

Patient categories	No. of patients receiving— Methotrexate alone	Methotrexate/daunorubicin	Total No. of patients
Completely evaluable	30	29	59
Partially evaluable	1	3	4
Not evaluable*	3	4	7
Total No. of patients	34	36	70

*One patient was lost to followup; six patients were nonevaluable due to protocol violation.

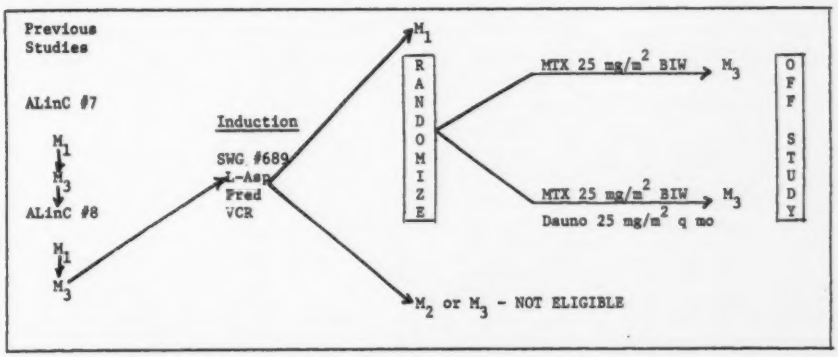

FIGURE 1.—Phase III evaluation of methotrexate (MTX) and daunorubicin (Dauno) in the treatment of ALL (Southwest Oncology Group study No. 675).

daunorubicin regimen the median was 291 days (21 patients).

The addition of a single monthly dose of daunorubicin (given iv) to the methotrexate regimen (given orally) did not interfere with the scheduled doses of methotrexate given orally (table 2). Patients receiving the methotrexate-alone regimen received 89.8% of the scheduled methotrexate therapy and patients receiving the methotrexate/daunorubicin regimen received 88.6% of the scheduled methotrexate therapy. Of the scheduled doses of daunorubicin, 72.8% was given.

The most common cause for omission of methotrexate therapy was stomatitis (table 3). Meningeal leukemia and intrathecal methotrexate therapy resulted in interruption of oral methotrexate therapy. Liver toxicity, fever with abdominal pain, and nonpulmonary infections caused a greater number of therapy deletions in the methotrexate-alone regimen, while pneumonia and bone marrow toxicity caused more therapy deletions in the methotrexate/daunorubicin regimen.

Myelosuppression was the most frequent side effect observed in this protocol (table 4), but there was no significant difference in the incidence of drug-related toxicity between the two maintenance regimens.

DISCUSSION

In this study, the addition of daunorubicin (25 mg/m² every 4 weeks) to the maximum tolerated dose of methotrexate given orally twice weekly was neither synergistic, additive, nor antagonistic. The Acute Leukemia Group B (ALGB) has also evaluated reinforcement therapy with daunorubicin (9). The ALGB compared maintenance reinforcement therapy with vincristine and prednisone to vincristine and prednisone plus daunorubicin. The relapse

TABLE 2.—Evaluation of therapy*

Chemotherapy	No. of doses—			Percent of scheduled drug given
	Omitted	Given	Scheduled	
Methotrexate alone	151	1336	1487	89.8
Methotrexate/ daunorubicin	180 47	1408 126	1588 173	88.6 72.8

*Evaluable patients only.

TABLE 3.—Primary causes for omission of therapy*

Cause	No. of incidents in which therapy was omitted		
	Metho-trexate alone	Methotrexate/daunorubicin	
		Methotrexate	Daunorubicin
Stomatitis	66	69	2
Concurrent intrathecal therapy and/or meningeal leukemia	26	39	0
Pneumonia	7	32	25
Liver toxicity	14	4	0
Bone marrow toxicity	2	8	7
Fever and/or abdominal pain	8	4	0
Infections (nonpulmonary)	4	0	0
Other	23	24	13
Total	150	180	47

*Evaluable patients only.

TABLE 4.—Incidence of drug-related toxicity*

Toxic effect	No. of patients with toxicity/ total No. of patients	
	Methotrexate alone	Methotrexate/ daunorubicin
Stomatitis	11/30	12/29
Pneumonia	3/30	4/29
Severe neutropenia (<1000 cells/mm³)	11/30	10/29
Mild neutropenia (<2000 cells/mm³)	12/30	13/29
Severe thrombocytopenia (<100,000 platelets/mm³)	8/30	11/29
Mild thrombocytopenia (<200,000 platelets/mm³)	13/30	8/29
Liver toxicity	3/30	1/29

*Evaluable patients only.

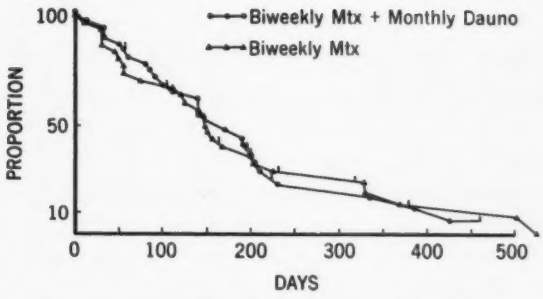

FIGURE 2.—Distribution of remission times for the two maintenance regimens.

rates were similar for both treatment regimens and daunorubicin was judged to be of no value in prolonging the duration of the first remission.

Daunorubicin is a closely related derivative of adriamycin (NSC-123127), an agent with a broad spectrum of anticancer activity. Because this study indicates that both methotrexate and daunorubicin can be given concomitantly, despite the fact that both agents are myelosuppressive, it might be assumed that methotrexate and adriamycin could also be used in combination with a similar lack of additive toxicity. Although this combination of methotrexate and daunorubicin was ineffective in the treatment of ALL, it might be of value in other clinical situations in which both methotrexate and daunorubicin (or adriamycin) are known to be effective as single agents (such as in the treatment of squamous cell carcinoma of the head and neck) (2).

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Hepatic Arterial Infusion for Liver Metastases From Colon Cancer: Comparison of Mitomycin C (NSC-26980) and 5-Fluorouracil (NSC-19893) [1,2,3]

I. E. Fortuny, A. Theologides, and B. J. Kennedy [4,5]

SUMMARY

This study compares the effectiveness of hepatic arterial infusion of mitomycin C with that of 5-fluorouracil in the management of carcinoma of the colon metastatic to the liver. A higher tumor response rate was obtained with 5-FU (ten of 12 patients) than with mitomycin C (six of 13 patients). The patients treated with mitomycin C had a median survival time of 9.5 months compared to 12 months for those receiving 5-FU, but this survival is not much different from that of the untreated historical controls. Femoral artery thrombosis requiring surgical thrombectomy developed in five of the 30 catheterized patients.

[Cancer Chemother Rep 59:401–404, 1975]

Percutaneous femoral or brachial artery catheterization for selective liver chemotherapy through the abdominal aorta or the hepatic artery has become a widely used procedure (1–3). The use of the Fenwald closed pressure system makes continuous and prolonged intra-arterial infusion chemotherapy easily applicable (2,3). With this approach more drug is introduced into the tumor due to the differential in the blood supply of the tumor and the liver, thus potentially resulting in a higher therapeutic effect (4–7). The intra-arterial administration of fluorinated pyrimidines (1–3) and alkylating agents (1,6) to liver tumors has proven to be tolerable and effective in inducing tumor shrinkage. However, the significant morbidity associated with this therapeutic approach should be considered prior to the catheterization.

Considerable information is available regarding the intra-arterial administration of 5-FU. The present study was designed to compare the effectiveness of hepatic arterial infusion of mitomycin C to that of 5-FU in cancer of the colon metastatic to the liver.

PATIENTS AND METHODS

Thirty patients with adenocarcinoma of the colon metastatic to the liver were randomized to receive either 5-FU or mitomycin C by hepatic arterial infusion (table 1). Nine of the patients were found to have biopsy-proven liver metastasis at the time of surgical resection of the primary lesion: four of these patients re-

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[2] Supported by Public Health Service grants CA-08101, CA-05158, and CA-08832 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Mitomycin C: CAS reg. No. 50-07-7.

5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU.

Both drugs were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

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[5] *Reprint requests to*: Athanasios Theologides, MD, PhD, Section of Medical Oncology, Department of Medicine, University of Minnesota School of Medicine, Box 286, University Hospitals, Minneapolis, Minn 55455.

TABLE 1.—Hepatic arterial chemotherapy for colon cancer metastatic to the liver

Patients—	No. of patients receiving— Mitomycin C	5-FU
Entered in study	15	15
Excluded (incomplete infusion)	2	3
Evaluable	13	12

ceived mitomycin C and five received 5-FU. The remainder of the patients also had recurrent biopsy-proven metastatic tumor involving the liver; however, in these 21 patients the median free interval between primary surgery and the occurrence of liver metastasis was 26 months.

Mitomycin C was administered at a total dose of 1 mg/kg of body weight in divided doses of 15 mg each. Each dose was infused over a 3–4-hour period at 12-hour intervals for 2–3 days. 5-FU was infused at a daily dose of 25 mg/kg over a 24-hour period. A total of 12 g constituted a course. The daily dose of these drugs was diluted in 1000–1500 ml of 5% D/W and 3000 units of heparin was added. Maintenance chemotherapy with the respective drug was continued after the completion of the original infusion (5 mg of mitomycin C given intravenously [iv] or 1 g of 5-FU given iv every week) as long as the weekly leukocyte count remained over 2500 cells/mm^3 and the platelet count remained over 100,000 platelets/mm^3. The arterial infusion was repeated every 8–10 weeks if objective response was documented or if significant subjective improvement was observed. Demonstration of new metastatic lesions, increase in liver size, or evidence of progression on liver scan required discontinuation of the study.

The hepatic artery was catheterized via a percutaneous femoral artery puncture, and the infusion was maintained by the Fenwald closed pressure system by the method previously described (1–3). Five of the patients had femoral artery thrombosis during the first 48 hours of the infusion which required surgical thrombectomy; these patients were excluded from the analysis of the therapeutic effects since they did not receive adequate chemotherapy by this method.

Leukocyte count, platelet count, prothrombin time, partial thromboplastin time, serum glutamic oxaloacetic transaminase (SGOT), ornithine carbamoyl transferase (OCT), alkaline phosphate, lactic dehydrogenase (LDH), and bilirubin were determined before and every 3 days during the period of infusion.

An objective response was defined as either a 50% or greater decrease in liver size (the size determined by measuring the liver below the costal margin and the liver span on percussion, both measurements taken at the midclavicular line), or as a 50% or greater change in the number and/or size of hepatic metastases as determined by radioactive technetium sulfur colloid (technetium 99) liver scan. Complete objective response consisted of a nonpalpable liver and a normal liver scan. The patients were evaluated before and 2 weeks after each infusion course.

Median survival was calculated from the time of histologic confirmation of liver metastasis which was also approximately the time of initiation of the infusion therapy.

RESULTS

Twenty-five patients completed at least the first course of infusion and were evaluable. Thirteen received mitomycin C and 12 received 5-FU. A total of 92 infusions were given, 17 patients receiving more than one course. The toxicity of both drugs was minimal; for mitomycin C a fever of 100–101° F occurred after the first three doses and lysed spontaneously. Minor changes were noted in the serial determinations of leukocyte count, platelet count, prothrombin time, and plasma thromboplastin time, but no significant leukopenia or thrombocytopenia was observed. Moderate elevations of SGOT, OCT, and LDH were transient and returned to normal 1 week after the infusion was completed.

There were no complete responses in either group. Sixteen patients had a partial response lasting from 1 to 20 months with a median duration of 8.5 months. The liver span below the costal margin in these patients decreased 50% or more; however, the liver scans remained essentially unchanged in most responding pa-

tients. Six of these patients received mitomycin C and ten received 5-FU (table 2).

The median survival time for the 16 responders was 12 months (table 2). The patients responding to mitomycin C had a median survival time of 11.5 months, compared to 12.5 months for those responding to 5-FU. The nine patients who failed to respond to a complete infusion course and the five patients excluded because of thrombosis after catheter placement had a median survival of 4.5 months.

Patients with synchronous liver metastasis found at the time of initial surgical resection of the bowel lesion had a comparable response to both drug regimens. Those treated with 5-FU had a median survival time of 16 months (range, 8–32 months) while those receiving mitomycin C had an 11-month median survival time (range, 7–13 months). Patients with metachronous liver metastasis occurring after the free interval from the time of the primary lesion resection showed a median survival time of 6 and 9 months respectively from infusion of 5-FU and mitomycin C.

DISCUSSION

Accurate evaluation of the objective response to therapy for metastatic tumors involving the liver is difficult. Evaluation of the liver size is of relative accuracy because of poor reproducibility. Serial determination of liver function does not always correlate with objective response. The use of isotope scanning of the liver with technetium sulfur colloid is of limited value in determining the effectiveness of treatment. This became obvious in two of our patients whose livers markedly regressed in size on physical examination while the scan failed to show any significant changes in the tumor lesions.

Objective response rates to the systemic administration of 5-FU to patients with colon tumors involving the liver are usually cited as 15%–50%, but a few reports of higher percentages have also appeared. In one study, 5-FU given orally was effective in producing complete or partial responses in 11 of 14 patients with metastatic colorectal cancer involving the liver (9). Intra-arterial administration of 5-FU resulted in roughly 40%–70% objective responses (1–3,7,8). Therefore, there is significant overlap in the response rates between systemic and intrahepatic 5-FU administration for liver metastasis from colon cancer.

Survival is the ultimate parameter for comparing the results of different chemotherapeutic approaches used in the treatment of colon carcinoma involving the liver. Patients who undergo primary resection and are found to have liver metastasis, but receive no subsequent chemotherapy, have shown a mean and median survival time of approximately 7–13 months (10–13). After intra-arterial infusion of 5-FU, the median survival time of responders ranged from 7 to 15 months (1–3,7). The median survival time in this study for patients responding to treatment was approximately 12 months for both drugs. The median survival time of nonresponders was 3 months for 5-FU and 4.5 months for mitomycin C.

Although in our study there is a suggestive finding that patients with synchronous liver metastasis had a longer median survival time

TABLE 2.—Response and survival with hepatic arterial chemotherapy

	Treatment	
	Mitomycin C	5-FU
No. of patients treated	13	12
No. of patients with—		
Complete response	0	0
Partial response	6	10
Median survival in mos (range)		
All patients	9 (2–13)	12 (3–32)
Responders	11.5 (9–13)	12.5 (4–32)
Nonresponders	4.5 (2–7)	3 (2–5)

than those with metachronous metastasis, this was not the case in another study (14).

Mitomycin C produces partial responses in colon cancer involving the liver, but the rate and duration of response may be inferior to those of 5-FU delivered through an infusion in the hepatic artery. However, the survival times of the responders and nonresponders were comparable for both drugs and the overall survival of these patients was not different from that of the historical untreated control patients who had cancer of the colon metastatic to the liver.

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Adriamycin (NSC-123127) Therapy for Advanced Gastrointestinal Cancer [1,2,3]

Stephen Frytak, Charles G. Moertel, Allan J. Schutt, Richard G. Hahn, and Richard J. Reitemeier [4,5]

SUMMARY

Sixty-nine patients with advanced gastrointestinal carcinomas were given adriamycin intravenously at a dose level of 40–75 mg/m² once every 3 weeks. Toxic effects included nausea, vomiting, diarrhea, stomatitis, alopecia, leukopenia, thrombocytopenia, and minor ECG changes. There was a slight trend toward more severe leukopenia in patients with markedly abnormal liver function tests (serum glutamic oxaloacetic transaminase and alkaline phosphatase). Of the 57 patients with colorectal cancer treated with adriamycin, four (7%) showed partial objective responses. In a controlled comparison of adriamycin versus 5-fluorouracil (5-FU) in patients with previously untreated large bowel carcinoma, three of 23 patients (13%) receiving adriamycin showed partial objective responses as compared with six of 25 patients (24%) receiving 5-FU. The median duration of response with adriamycin was 3 months compared to over 6 months with 5-FU. Four of eight patients with gastric carcinoma showed partial objective responses. No responses were noted in a small number of patients with pancreatic and gallbladder carcinomas. Adriamycin would not seem to have any role in the treatment of advanced colorectal carcinoma. Our results, however, would justify further evaluation of this agent in gastric carcinoma.

[Cancer Chemother Rep 59:405–409, 1975]

Adriamycin has been one of the most promising antineoplastic agents undergoing investigation in the treatment of solid tumors. Previous experience with this antibiotic derivative of *Streptomyces peucetius* has shown therapeutic activity in a broad spectrum of malignant neoplasms. The response rate reported in gastrointestinal carcinomas has been variable, ranging from zero to 20%, with an overall response rate of 9% among 58 patients from five series (1).

Our study was predominantly designed to look at the antineoplastic activity of adriamycin in large bowel carcinoma, utilizing an ambulatory patient population containing a significant number of patients not previously exposed to cytotoxic drugs. It was also our aim to compare the therapeutic activity of adriamycin with

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[2] Supported by contract 70-2066 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Adriamycin and all other drugs used in this study were supplied by Cancer Therapy Evaluation, DCT, NCI.

[4] Mayo Clinic and Mayo Foundation, Rochester, Minn.

[5] *Reprint requests to:* Stephen Frytak, MD, Mayo Clinic, Rochester, Minn 55901.

that of 5-fluorouracil (5-FU)[6] and to look in a preliminary fashion at the activity of adriamycin against other gastrointestinal neoplasms.

MATERIALS AND METHODS

Patient Selection

All patients included in the study had histologic proof of unresectable adenocarcinoma of the gastrointestinal tract. Entry into the study required each patient to be ambulatory and to be maintaining a reasonable state of nutrition. Contraindications to selection were surgical therapy, radiotherapy, or treatment with cytotoxic drugs within the preceding 30 days; present evidence of active cardiac disease; leukopenia ($<$ 4000 cells/mm^3); thrombocytopenia ($<$ 130,000 cells/mm^3); or an active infectious process of major clinical significance.

A total of 69 patients with gastrointestinal cancer were treated with adriamycin. There were 39 men and 30 women. The mean age was 59 years with a range of 28–77 years. Twenty-six of the patients had not received prior chemotherapy while 43 had been treated with a variety of agents, primarily 5-FU or a nitrosourea (table 1). Ten patients had received radiation therapy. Our comparative study with 5-FU involved 48 previously untreated patients with colorectal carcinoma who were randomly assigned to adriamycin or 5-FU programs.

Treatment Methods

Adriamycin was supplied as a red powder in 10-mg vials. Five ml of physiologic saline was used as the diluent to give a concentration of 2 mg/ml. The drug was administered as a single dose through the tubing of a freely running intravenous (iv) infusion.

Originally, we had planned to use a single injection dose of 75 mg/m^2; however, because of severe leukopenia (range, 750–1600 cells/mm^3) in the first six patients treated at this level, we elected to complete our study at a dose level of 60 mg/m^2. If clinical jaundice or an increase in the direct reacting bilirubin was present, the starting dose of adriamycin was reduced to 40 mg/m^2. The total cumulative dose of adriamycin was set at 550 mg/m^2 because of the increased frequency of toxic myocardiopathy above this level.

Each patient was examined before each injection of adriamycin. Prior to initial treatment the following tests and determinations were obtained: hemoglobin value, leukocyte and platelet counts, alkaline phosphatase (AP), serum glutamic oxaloacetic transaminase (SGOT), calcium, bilirubin, creatinine, urinalysis, ECG, and chest roentgenogram. Leukocyte and platelet counts were obtained twice weekly for 3 weeks after treatment and an ECG was obtained before administering each subsequent treatment. If their condition permitted, the patients were re-treated at 3 and 6 weeks. Those patients whose conditions remained objectively stable or who showed objective improvement at 6 weeks were maintained on treatment every 4 weeks. Doses of adriamycin in subsequent courses were adjusted to produce definite but clinically tolerable toxicity.

For our controlled evaluation, 5-FU was administered in a 5-day course randomly assigned to be given either as a rapid iv injection at a dose level of 13.5 mg/kg/day or orally at a dose level of 20 mg/kg/day.

In evaluating an objective response, we

[6] 5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

TABLE 1.—Sites of origin of gastrointestinal carcinomas and prior treatment status of patients

Site	Total No. of patients	No. of patients with prior chemotherapy	No. of patients with no prior chemotherapy
Colorectal	57	32	25
Stomach	8	8	0
Pancreas	3	3	0
Gallbladder	1	0	1

employed the following criteria: (*a*) a reduction by at least 50% of the product of the longest perpendicular diameters of the most clearly measurable area of known malignant disease; (*b*) if malignant hepatomegaly was the indicator, a reduction by at least 30% of the sum of the liver measurements below each costal margin and xiphoid process; (*c*) no increase in the size of the other areas of malignant disease; (*d*) no new areas of malignant disease; and (*e*) maintenance of the regression for at least 6 weeks after the start of therapy.

RESULTS

Toxicity

The toxic reactions noted in the adriamycin-treated patients are listed in table 2.

Nausea and vomiting, although relatively frequent, usually lasted no longer than 36 hours after treatment. Phenothiazines, used prior to therapy, were useful in ameliorating these side effects. Stomatitis was uncommonly observed and was usually mild in degree. Alopecia, including facial, axillary, and pubic hair, was noted, at least in moderate degree, in the vast majority of the patients treated. Liver and kidney function tests were not perceptibly affected by adriamycin therapy. No serious cardiac toxicity was noted in the 69 patients treated at the 60-mg/m² dose level. Six of the patients had minor ST-T changes on their ECGs, but arrhythmias, conduction defects, or congestive failure were not seen in any of the patients.

TABLE 2.—Toxic reactions observed in 69 adriamycin-treated patients

Toxic reaction	Observed frequency*
Nausea	29/69
Vomiting	16/69
Stomatitis	10/69
Alopecia	68/69
Diarrhea (>5 watery stools/day)	4/69
ECG abnormalities	6/69
Leukopenia	
(2000–4000 cells/mm³)	32/69
(<2000 cells/mm³)	18/69
Thrombocytopenia	
(50,000–130,000 platelets/mm³)	13/69
(<50,000 platelets/mm³)	1/69

*No. of patients having toxic reactions/total No. of patients.

Hematologic toxicity was similar to that reported by others who have used a comparable drug dose. The mean nadir occurred on Day 12 for leukopenia and on Day 10 for thrombocytopenia. Leukopenia was noted to occur approximately three times more frequently than thrombocytopenia; however, two patients had thrombocytopenia without leukopenia.

Because adriamycin is largely metabolized in the liver, we were concerned with the possible adverse effects which impaired hepatic function might have on the drug's toxicity. We studied this matter in more detail using our nonjaundiced patients who had initially been treated at a dose level of 60 mg/m². Our experience with this problem in relation to leukopenia is summarized in figure 1. Although the median nadir of leukopenia was lower in patients with significant abnormalities in SGOT or AP levels, the dose of 60 mg/m² did not produce prohibitive hematologic toxicity. The number of adriamycin-treated patients in whom thrombocytopenia or stomatitis developed was too small to make any definite conclusions regarding increased incidence of these conditions with abnormal liver function. In the absence of jaundice, we saw no need to reduce the dose for the patient with impaired enzyme tests of liver function. Indeed, the majority of patients with normal SGOT and AP values had either minimal or no toxicity, and presumably would have tolerated adriamycin at a dose higher than 60 mg/m².

The ten patients with prior radiation therapy did not appear more susceptible to increased drug toxicity.

Therapeutic Response

The objective response rate to adriamycin of the patients with various gastrointestinal neoplasms is noted in table 3.

Of the 69 patients treated with adriamycin, eight (12%) showed objective partial regressions. All objective responses were noted within the first 6 weeks. Although this overall response

TABLE 3.—Objective response rate to adriamycin at 10 weeks of patients with gastrointestinal neoplasms

Type of neoplasm	No. of patients achieving partial objective responses*	
	No prior chemotherapy	Prior chemotherapy
Colorectal	3/25	1/32
Stomach	0/0	4/8
Pancreas	0/0	0/3
Gallbladder	0/1	0/0

*No. of patients achieving partial objective responses/total No. of patients.

rate is poor, some interesting findings are noted when specific tumor types are analyzed separately.

The patients with colorectal carcinoma comprised our largest group and the response rate to adriamycin was 7% (four of 57 patients). One patient who had failed to respond to 5-FU and to radiation therapy had an objective regression that was maintained for 6 months. He was treated with the maximum dose of 550 mg/m² and, thereafter, was treated with another agent. Three other patients who had not received prior treatment had objective partial regressions that lasted 3, 3, and 3½ months, respectively. The results of the controlled comparison of adriamycin and 5-FU in previously untreated patients are shown in table 4. Six of 25 5-FU-treated patients (24%) showed objective responses which were maintained at least 10 weeks as compared with three of 23 patients (13%) treated with adriamycin ($P \simeq 0.3$). Of the six objective responses to 5-FU, four occurred in patients who received the drug iv and two occurred in patients who received the drug orally. The median duration of response in the adriamycin-treated patients was only 3 months as compared to a median duration in the 5-FU-treated patients which will be at least 6 months. One patient in the 5-FU program is still maintaining an objective response.

Four of eight patients with gastric carcinomas showed objective partial responses to adriamycin. These responses have lasted from 2½ to 10½ months. In addition to these, there was a fifth patient with an objective response noted at 3 weeks but in whom progressive disease occurred at 6 weeks. No responses were noted in those few patients entered with gallbladder or pancreatic carcinoma.

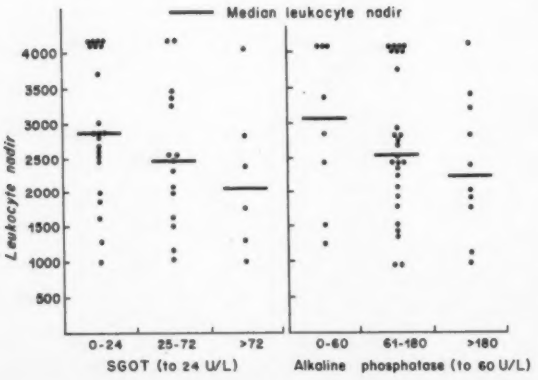

FIGURE 1.—Liver function values and subsequent leukocyte nadirs after first dose of adriamycin at 60 mg/m².

DISCUSSION

With the methods employed in this study, adriamycin showed only a 7% overall response rate in patients with colorectal carcinoma. If we consider only the previously untreated patients, a response rate of 12.5% is noted, but the duration of response was very short. Although some antineoplastic activity was demonstrated in these patients, the magnitude of this activity was small. Our results confirm the overall impression that adriamycin has no significant role in the treatment of colorectal carcinoma.

The number of patients with gastric carcinoma included in this study was small. We are, however, impressed with the 50% response

TABLE 4.—Controlled comparison of adriamycin versus 5-FU against colorectal carcinomas

Drug	No. of patients achieving partial objective responses*	Duration of response (mos)
Adriamycin	3/23	3, 3, 3½
5-FU	6/25	2½, 4½+, 6, 6, 6, 8+

*No. of patients achieving partial objective responses/total No. of patients receiving drugs.

rate in this group of patients, all of whom had previous exposure to chemotherapy. More data should be obtained to properly evaluate adriamycin's overall clinical effectiveness against gastric carcinoma. Consideration might also be given to incorporating adriamycin into combination chemotherapy regimens for patients with gastric carcinomas.

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Intensive Cyclophosphamide (NSC-26271) Therapy for Solid Tumors [1,2,3]

G. M. Mullins [4,5] and M. Colvin [4,6]

SUMMARY

Cyclophosphamide was given in two dose schedules to 25 patients with a variety of nonlymphoid solid tumors. Eleven patients were given 18 courses of cyclophosphamide at a total dose of 60 mg/kg. Sixteen patients received 26 courses at a total dose of 100 mg/kg. Two patients were treated with both regimens. Partial responses were achieved in two patients treated with the 60-mg/kg dose of cyclophosphamide. One of these patients had osteogenic sarcoma and the other had renal carcinoma. The higher dose also produced two partial responses, one in a patient with anaplastic carcinoma of the lung and the other in a patient with embryonal testicular carcinoma. Mean leukocyte counts fell to a nadir of 1400 cells/mm^3 after 60 mg/kg, while they dropped to below 1000 cells/mm^3 for 5 days after 100 mg/kg of cyclophosphamide. Mean platelet counts remained above 150,000 platelets/mm^3 after both cyclophosphamide schedules. Infective complications were documented after three of the 18 courses at 60 mg/kg and after ten of the 26 courses at 100 mg/kg. In the latter group, there were three episodes of bacteremia, including one death from pseudomonas sepsis. Nonhematologic toxicity noted with the 100-mg/kg dose of cyclophosphamide included rare instances of electrocardiogram changes and serum enzyme alterations compatible with myocardial toxicity. The intensive cyclophosphamide therapy did not appear to result in an increased antitumor response in malignancies usually considered to be refractory to alkylating agents.

[Cancer Chemother Rep 59:411–419, 1975]

Cyclophosphamide has been used more frequently in daily oral or weekly parenteral schedules than in intensive regimens which may be repeated following hematopoietic recovery. The studies of Bruce et al (1) and Skipper et al (2) in animal models suggest that large doses of cyclophosphamide may produce greater therapeutic benefit. Bruce et al (1), using a spleen colony assay system, found a greater

[1] Received Sept 6, 1974; accepted Nov 20, 1974.

[2] Supported by Public Health Service grant CA-06973 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,-3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate. Supplied by Mead Johnson Laboratories, Evansville, Ind.

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TABLE 1.—Patients receiving cyclophosphamide

Patient No.	Age/ sex	Tumor type	Prior therapy	Dose of cyclophosphamide (mg/kg)	No. of courses	Response*	Duration of response (mos)	Survival after beginning cyclophosphamide (mos)
1	23/F	Embryonal rhabdomyosarcoma	Irradiation	60	2	NR	—	18
2	65/M	Anaplastic carcinoma, lung	None	60	1	NR	—	2
3	53/M	Mesothelioma, pleura	Irradiation	60	1	NR	—	4
4	68/M	Squamous carcinoma, lung	None	60	1	DNM	—	8
5	63/M	Adenocarcinoma, lung	None	60	1	NR	—	3
6	61/M	Squamous carcinoma, lung	None	60	1	NR	—	2
7	59/M	Adenocarcinoma, kidney	None	60	2	NR	—	4
8	26/M	Islet cell carcinoma, pancreas	Streptozotocin†	60	1	NR	—	18
9	26/M	Embryonal cell carcinoma, mediastinum	Irradiation	60	1	NR	—	3
10	16/F	Osteogenic sarcoma	Irradiation	60–100	7	PR	1	19
11	54/M	Adenocarcinoma, kidney	None	60–100	4	PR	4	20
12	52/M	Anaplastic carcinoma, lung	None	100	1	PR	2	3
13	26/M	Embryonal carcinoma, testis	Multiple chemotherapy	100	3	PR	2	5
14	47/F	Anaplastic carcinoma, lung	None	100	2	NR	—	17
15	44/M	Adenocarcinoma, lung	None	100	2	NR	—	13
16	60/F	Adenocarcinoma, ovary	None	100	2	NR	—	6
17	63/F	Squamous carcinoma, lung	None	100	2	NR	—	7
18	39/M	Anaplastic carcinoma, lung	Chemotherapy	100	1	NR	—	4
19	16/F	Anaplastic carcinoma, primary unknown	None	100	2	NR	—	5

TABLE 1.—Patients receiving cyclophosphamide—Continued

Patient No.	Age/ sex	Tumor type	Prior therapy	Dose of cyclophos-phamide (mg/kg)	No. of courses	Re-sponse*	Duration of response (mos)	Survival after beginning cyclophos-phamide (mos)
20	38/F	Anaplastic sarcoma, liver	ThioTEPA‡	100	1	NR	—	5
21	59/M	Melanoma	Chemotherapy	100	1	NR	—	3
22	74/M	Adenocarcinoma, kidney	Chemotherapy	100	1	NR	—	13
23	62/F	Adenocarcinoma, colon	Chemotherapy	100	1	NR	—	17
24	55/F	Carcino-sarcoma, ovary	None	100	2	DNM	—	11
25	50/F	Anaplastic carcinoma, lung	None	100	1	NR	—	<1

*PR = partial response; NR = no response; DNM = disease nonmeasurable.
†Streptozotocin: NSC-85998.
‡ThioTEPA: NSC-6396; CAS reg. No. 52-24-4; phosphine sulfide, tris(1-aziridinyl)-.

reduction of colony-forming units of transplanted AKR lymphoma cells than of hematopoietic stem cells with increasing doses of cyclophosphamide. The difference is presumed to be due to the nonproliferative state of a large proportion of hematopoietic stem cells which consequently tend to be less susceptible to the effects of many cytotoxic agents.

The present study was designed to examine the therapeutic and toxic effects of cyclophosphamide given in the two dose schedules of 60 and 100 mg/kg.

PATIENTS AND METHODS

The 25 patients entered in this protocol had various nonlymphoid metastatic solid malignancies (table 1). They had an estimated survival of at least 3 months and their ages ranged from 16 to 74 years with a mean of 48 years. Other forms of treatment had not been administered within 2 months preceding cyclophosphamide therapy. Fourteen patients had not received any prior treatment. Seven patients had previously been given chemotherapy with various other agents.

Patients were randomly assigned to one of two treatment categories. One group was given cyclophosphamide at a dose of 60 mg/kg while the other received 100 mg/kg. Total doses were divided into four equal doses which were given on four consecutive days by intravenous (iv) infusion. The first day of cyclophosphamide administration was considered Day 1 of the course of therapy.

Two to 3 liters of fluid were given iv on Days 1–5 of therapy for the prevention of hemorrhagic cystitis. If leukopenia of less than 1000 cells/mm^3 occurred, simple reverse barrier isolation precautions were instituted. Broad-spectrum systemic antibiotic coverage was given if fever of 101° F or greater or some other indication of infection developed during the period of cyclophosphamide-induced myelodepression. In most instances the antibiotic therapy used for fever of undetermined origin was a combination of carbenicillin and gentamicin. Platelet transfusions were administered for thrombocytopenia of less than 20,000 platelets/mm^3. Cardiac toxicity was evaluated in patients receiving 100 mg/kg of cyclophosphamide by serial electrocardiograms (EKGs), by estimations of serum glutamic oxaloacetic transaminase (SGOT) and creatine phosphokinase (CPK), and by frequent physical examinations.

RESULTS

Tumor Responses

A partial response was considered to be a

reduction of 50% or more in measurable tumor size, accompanied by subjective improvement and lasting 1 month or longer. Therapeutic responses are summarized in table 1.

Two of ten patients with objectively measurable disease who were treated with cyclophosphamide at a dose of 60 mg/kg had a partial response. These two cases are described below as case reports. Two other patients given this cyclophosphamide dosage and classed as nonresponding in table 1 (patients Nos. 1 and 2) showed tumor regression which was definite but did not amount to a 50% reduction in measurable tumor mass.

Fifteen patients with evaluable disease received 100 mg/kg of cyclophosphamide. Partial responses were achieved in two of these patients. One patient had anaplastic carcinoma of the lung. When his disease progressed 2 months later, his general condition was considered unsuitable for further intensive cyclophosphamide therapy. The second patient who showed a partial response had embryonal carcinoma of the testis. He had previously failed to obtain a remission with combination chemotherapy and later with mithramycin [7] given alone. After one course of cyclophosphamide at a dose of 100 mg/kg, serial chest roentgenograms revealed a virtually complete disappearance of multiple lung metastases, but the patient's gonadotropin titer remained elevated. Recurrent pulmonary disease became evident after 2 months. Two further courses of cyclophosphamide at a dose of 100 mg/kg produced responses which were dramatic but of shorter duration. Four other patients who were given 100 mg/kg of cyclophosphamide (patient No. 10 and Nos. 14–16 in table 1) had significant, but less than partial, responses.

Case Reports

Patient No. 10.—Patient No. 10 was a 16-year-old girl with osteogenic sarcoma of the distal right femur. She received 7000 rads to the tumor area and then underwent disarticulation at the hip. Eight months later lung metastases became evident. Pulmonary involvement progressed to almost complete opacification of the right hemithorax with mediastinal displacement and considerable respiratory dysfunction. Two courses of cyclophosphamide at doses of 60 mg/kg resulted in considerable improvement, and a partial response was achieved after a third course. Five weeks later, recurrent disease became apparent. Two further courses of cyclophosphamide at the same dose produced definite, but less than 50%, responses. In the hope that more intensive therapy would be more beneficial, the dose of cyclophosphamide was increased to 100 mg/kg. An antitumor effect not amounting to a partial response was seen with each of three courses of cyclophosphamide at this dosage. Toxicity was more marked with the more intensive regimen. Two of the three courses were complicated by infections, including one episode of pseudomonas bacteremia. Evidence suggestive of cyclophosphamide-induced cardiotoxicity was seen, with transient depression of the ST segment and depression of the T wave on EKG and elevation of serum CPK levels. The possibility of cumulative cyclophosphamide toxicity was considered. Subsequent combination chemotherapy did not arrest disease progression. She died 19 months after her initial course of cyclophosphamide.

Patient No. 11.—Patient No. 11, a 54-year-old man, had progressive dyspnea associated with a malignant pleural effusion and bilateral nodular pulmonary metastases. Nine years previously breast carcinoma had been discovered and treated successfully, with no evidence of recurrence in the interim. Renal carcinoma was found on nephrectomy. After initial chemotherapy with cyclophosphamide at a dose of 60 mg/kg, serial chest roentgenograms revealed virtual clearing of the lung metastases. The onset of the marked improvement was delayed for about 4 weeks. Partial response was sustained for 4 months. When advancing disease was documented, two further courses of cyclophosphamide at doses of 60 mg/kg again produced considerable improvement. His fourth and last course of cyclophosphamide consisted of 100 mg/kg. It was felt that he did not respond to the higher dose regimen. He was subsequently treated with a variety of chemotherapeutic agents used alone and in combination, but they did not produce a significant anti-

[7] Mithramycin: NSC-24559; CAS reg. No. 18378-89-7.

tumor effect. His survival time was 20 months after beginning cyclophosphamide therapy.

Toxicity

The mean changes in leukocyte and platelet counts and in hematocrit values in patients receiving 60 and 100 mg/kg of cyclophosphamide are shown in figure 1. The hematologic toxicity following the two dosage levels is summarized in table 2. The mean nadir of the white blood cell counts was 1400 cells/mm³ on Day 11 after 60 mg/kg of cyclophosphamide and 400 cells/mm³, also on Day 11, following cyclophosphamide at a dose of 100 mg/kg. Leukopenia of less than 1000 cells/mm³ was seen after ten of 18 courses of cyclophosphamide at a dose of 60 mg/kg and after all but two of 26 courses at the 100-mg/kg dose of cyclophosphamide. Average platelet counts remained above 150,000 platelets/mm³ after both cyclophosphamide regimens. Platelet counts of less than 100,000 platelets/mm³ were recorded in two patients after treatment with 60 mg/kg and in eight patients after treatment with 100 mg/kg. Two patients given the more intensive cyclophosphamide dosage required platelet transfusions for transient thrombocytopenia of less than 20,000 platelets/mm³.

Data on the frequency, onset, duration, and treatment of fevers and infections during the period of leukopenia are shown in table 3. Patients being considered for intensive cyclophosphamide therapy who were febrile were thoroughly evaluated for a possible site of infection, and only those in whom the temperature elevation was considered to be due to their malignant disease were entered on the protocol. One patient with undifferentiated carcinoma of the liver had intermittent fever from before the time of cyclophosphamide therapy and was considered nonevaluable (table 3). After 18 courses of 60 mg/kg of cyclophosphamide, fever of 101°F or greater developed in seven instances during the time of cyclophosphamide-induced myelodepression. In three instances local infections were identified while in the other four no cause could be identified. There were 19 episodes of fever following 25 evaluable courses of 100 mg/kg of cyclophosphamide. Seven of the 19 episodes were due to local infections and

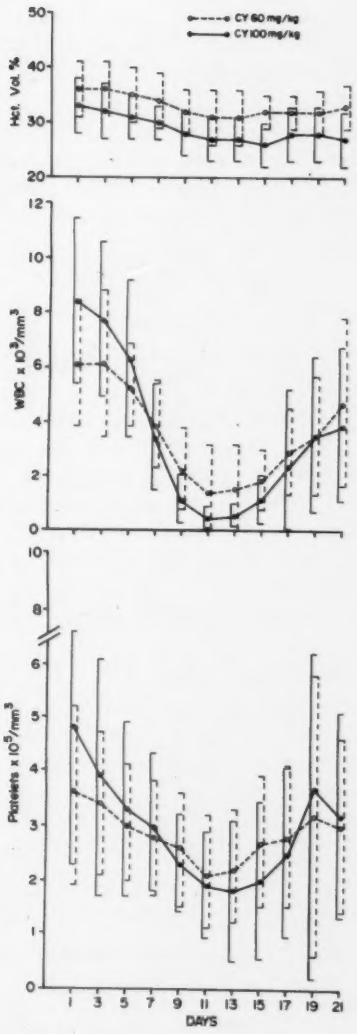

FIGURE 1.—Mean ± 1 SD changes in hematocrit levels and white blood cell and platelet counts in patients receiving 60 mg/kg (○------○) (18 courses) and 100 mg/kg (●——●) (26 courses) of cyclophosphamide.

three to bacteremia while the others remained of undetermined origin. One case of bacteremia and pneumonia due to pseudomonas was fatal. This was the only drug-related death in the series. Other infections responded well to appropriate antibiotic therapy. When all febrile episodes are considered together, irrespective of

TABLE 2.—Hematologic toxicity of cyclophosphamide

	Total dose (mg/kg)	
	60	100
No. of patient courses	18	26
White blood cell counts:		
No. of courses followed by counts <1000 cells/mm³	10	24
Mean nadir (cells/mm³ ± SD)	1400 ± 1700	400 ± 400
Platelet counts:		
No. of courses followed by counts <100,000 platelets/mm³	2	8
Mean nadir (platelets/mm³ ± SD)	214,000 ± 106,000	182,000 ± 130,000
Hematocrit levels:		
Mean maximum depression (vol % ± SD)	5.8 ± 3.2	8.3 ± 3.7

TABLE 3.—Fever and infection during cyclophosphamide-induced leukopenia

	Total dose (mg/kg)	
	60	100
Total No. of courses of therapy	18	26
No. of evaluable courses of therapy	18	25
No. of febrile episodes*	7	19
Mean day of onset of fever	12	9
Mean No. of days of fever	3.5	5
Mean No. of days on systemic antibiotic therapy	6.5	9
No. of localized infections	3	7
No. of bacteremias	0	3
No. of instances of fever of undetermined origin	4	9

*Fever ≥101°F.

their etiology, patients receiving cyclophosphamide at a dose of 60 mg/kg who became febrile had a slightly shorter average duration of fever and a somewhat more brief mean period of systemic antibiotic therapy than patients treated with 100 mg/kg of cyclophosphamide.

Table 4 summarizes the nonhematologic toxicity of the 60- and 100-mg/kg dose schedules of cyclophosphamide. Gastrointestinal upset was very common but seldom lasted longer than a few days. Diarrhea occurred at the time of therapy in several patients. Transient severe or total alopecia was seen in most patients after both cyclophosphamide regimens. Hemorrhagic cystitis was not a major problem. Increased numbers of red blood cells on microscopic examination of serial-centrifuged specimens of fresh urine were noted after three of 15 courses at the lower dose and after nine of 23 courses at the higher cyclophosphamide dose. Microhematuria was usually of brief duration. Gross hematuria and symptomatic cystitis lasting

longer than 1 week occurred in only one instance in a patient given 100 mg/kg of cyclophosphamide.

Patients' weights were followed closely during and for some days after the 4 days of cyclophosphamide therapy. A weight increase of or exceeding 2 kg was only documented once. Findings compatible with the syndrome of inappropriate antidiuretic hormone secretion have been described in patients receiving intensive cyclophosphamide therapy (3). The frequency of abnormalities in the concentrations of serum enzymes sensitive to myocardial damage in patients receiving 100 mg/kg of cyclophosphamide is indicated in table 4. Only two patients treated with 100 mg/kg showed transient EKG changes at the time of drug administration. The changes consisted of depression of the ST segment and flattening or inversion of the T wave without significant diminution in voltage. Repeated clinical examinations revealed no evidence of cardiac dysfunction. Cardiotoxicity, induced by high doses of cyclophosphamide, is discussed later.

DISCUSSION

This report describes the antitumor and toxic effects of cyclophosphamide given in the two dose schedules of 60 and 100 mg/kg. The therapeutic values of the two regimens cannot be adequately assessed or compared in this relatively small series of patients with a variety of solid tumor types. There were two partial responses after 60 mg/kg and two after 100 mg/kg of cyclophosphamide, in each case in a different type of tumor. The two case reports described are noteworthy in that both patients had partial responses after 60 mg/kg of cyclophosphamide and both were subsequently treated with the 100-mg/kg regimen. The higher dose produced a definite but less than partial response in one patient (No. 10) while it did not cause significant tumor resolution in the other patient (No. 11). The report of patient No. 10 illustrates the greater toxicity encountered at the 100-mg/kg dose of cyclophosphamide.

Cyclophosphamide given in doses of 60 mg/kg was generally well tolerated. Leukopenia was significant but transient, and infective complications were easily managed. As would be expected, the higher dose of 100 mg/kg produced more severe hematologic and also nonhematologic toxicity. Leukopenia was more severe, although not much more prolonged. Febrile episodes and documented infections were more common. The combination of carbenicillin and gentamicin, which was given in the majority of instances, has proved very effective in the management of episodes of fever of undetermined origin occurring during chemotherapeutically induced myelodepression in solid tumor patients (4). Thrombocytopenia was generally mild with both doses, and only two patients had a transient fall in platelet counts to less than 20,000 platelets/mm^3.

Nausea, with or without vomiting, and hematuria were more marked with 100 than with

TABLE 4.—Nonhematologic toxicity of cyclophosphamide

	Total dose (mg/kg)	
	60	100
No. of courses of therapy	18	26
No. of instances of—		
Nausea with or without vomiting	16/18*	25/26
Alopecia	16/18	26/26
Hematuria (microscopic with or without gross)	3/15	9/23
Weight gain ≥2 kg	0/16	1/20
SGOT elevation	—	7/20
CPK elevation	—	5/19
EKG changes	—	2/18

*No. of abnormal observations/No. of evaluable courses.

60 mg/kg of cyclophosphamide. Weight gain attributed to fluid retention was noted on only one occasion in a patient given 100 mg/kg of cyclophosphamide. Findings compatible with the syndrome of inappropriate release of antidiuretic hormone in patients given doses of cyclophosphamide of or exceeding 50 mg/kg/day have been reported by this group (3).

Myocardial toxicity has been found after large doses of cyclophosphamide and appears to be a major dose-limiting factor in intensive cyclophosphamide therapy. In the present series there were a small number of instances of transient EKG changes and alterations in serum enzymes compatible with myocardial damage in patients receiving 100 mg/kg of cyclophosphamide. There was no clinical evidence of cardiac dysfunction. Similar observations have been described by our group [8] and by Buckner et al (5) in patients given cyclophosphamide at doses of 120 mg/kg over 48 hours. Fatal cardiac failure caused by cyclophosphamide-induced myocardial necrosis was noted by Santos et al (6,7) in a patient given a total dose of 270 mg/kg and an allogeneic marrow transplant, and by Buckner et al (5) in a patient who received 240 mg/kg and a marrow autograft. Myocardial damage was recognized as a contributory cause of death in a patient given 120 mg/kg in a continuous 24-hour infusion (8). Animal studies have also provided evidence of cyclophosphamide-induced cardiac damage. Diffuse cardiac hemorrhage and necrosis were found in rhesus monkeys given doses of 240 mg/kg (9). EKG changes and myocarditis have been reported in dogs after 500 mg/kg of cyclophosphamide (10).

Experience with the use of cyclophosphamide in single doses of 50 and 100 mg/kg in solid tumor patients has indicated that the severity of leukopenia and the frequency of infective complications are approximately similar to those seen in the patients in this series who received comparable total doses given over 4 days.[9] When the total amount of cyclophosphamide was divided into four equal daily doses, cystitis tended to be less severe, the syndrome of inappropriate antidiuretic hormone release was not a problem, and cardiotoxicity was less evident.

Intensive cyclophosphamide therapy has been used at this center in a number of clinical investigations. Cyclophosphamide has been employed for its cytoreductive and immunosuppressive effects in bone marrow transplantation from HL-A identical siblings in hemolymphopoietic malignancies and bone marrow failure. The antitumor effects obtained and the satisfactory engraftment usually achieved have been well established (6,7,11). The transplant protocol involves the use of cyclophosphamide as the sole preparatory cytotoxic agent at a dose of 50 mg/kg daily for 4 days. Further cyclophosphamide has been given after transplant to control the severity of the graft-versus-host reaction. The therapeutic, toxic, and immunosuppressive effects of cyclophosphamide at a dose of 120 mg/kg have been examined in patients with various types of solid tumors.[10] Patients with acute nonlymphocytic leukemia have been randomized either to cyclophosphamide alone at a dose of 25 mg/kg daily for 4 days or to combination therapy comprising cyclophosphamide, cytosine arabinoside, and vincristine (12).[11] The complete remission rate to initial therapy was 20% with cyclophosphamide alone compared to 53% with the combination. Poor-risk patients with multiple myeloma and Waldenström's macroglobulinemia have been treated with cyclophosphamide at doses of 15 mg/kg on each of 4 successive days (13). Eleven of 22 patients showed a decrease of 50% or more in their serum or urinary M-type protein.

In the current study an increased antitumor response in solid tumors generally resistant to alkylating agents was not apparent. Poor responses to intensive cyclophosphamide therapy have also been found in other series of refractory tumors. Partial responses were rarely

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[8] Mullins GM, Anderson PN, and Santos GW. High dose cyclophosphamide therapy in solid tumors. Therapeutic, toxic and immunosuppressive effects. Manuscript submitted for publication.

[9] Colvin M. Unpublished observations.

[10] *See* footnote 8.

[11] Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

achieved in patients with malignant melanoma given doses ranging from 1500 mg/m² (40 mg/kg) to 1750 mg/m² of cyclophosphamide (14), and in patients with various nonresponsive nonlymphoid solid tumors treated with cyclophosphamide at doses of 60–120 mg/kg in one series (5) and 120 mg/kg in another.[10] On the other hand, good results have been reported in Burkitt's lymphoma (15) and lymphosarcoma (16) using cyclophosphamide at doses of 40 mg/kg. Very satisfactory responses were achieved in ovarian carcinoma with doses of 60–120 mg/kg (5). Further trials with intensive chemotherapy appear indicated in tumors known to be sensitive to cyclophosphamide and other alkylating agents.

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Prognosis of Diffuse (Non-Nodular) Non-Hodgkin's Lymphoma [1,2]

Richard A. Rudders and Darrell Hensleigh [3,4,5]

Brief Reports and Preliminary Communications

In 1956, Rappaport et al (1) emphasized that non-Hodgkin's lymphomas with a follicular (nodular) histologic pattern had a significantly better prognosis than those with a diffuse (non-nodular) pattern. More recently, Jones et al (2,3) and Schein et al (4) have applied this type of histologic classification to a retrospective analysis of their experience, and they have confirmed that a nodular histologic characteristic is a favorable prognostic determinant. Since in most large series a majority of patients with non-Hodgkin's lymphomas have a diffuse histologic pattern, this study was undertaken to define the overall prognosis of this less favorable group. Our report describes our experience in treating 33 patients with advanced diffuse non-Hodgkin's lymphomas.

MATERIALS AND METHODS

Thirty-three consecutive patients seen at the teaching hospitals of the Medical College of Wisconsin (Milwaukee County General Hospital and Veterans' Hospital-Wood) between July 1969 and July 1972 with the diagnosis of disseminated (stages III and IV) diffuse non-Hodgkin's lymphoma were studied. Biopsy material was available and was reviewed in all cases. We excluded all patients with a nodular histologic pattern of any degree, as well as those in whom the diagnosis of chronic lymphocytic leukemia was questionable.

Patients were staged according to the scheme proposed for Hodgkin's disease at Ann Arbor, Michigan (5). In addition to the usual staging procedures, bipedal lymphangiography, closed liver biopsy, and rarely a laparotomy were performed.

During this time patients at our institution were treated with either combination chemotherapy (COP)[6] or a single drug (cyclophosphamide). The COP regimen was a modification of the combination chemotherapy regimen reported by Hoogstraten et al (6) and consisted of cyclophosphamide (25 mg/kg) and vincristine (0.25 mg/kg) given intravenously (iv) once weekly for 6 weeks, and prednisone (1 mg/kg) given daily for 6 weeks. Responders were given continuous oral cyclophosphamide maintenance therapy (2 mg/kg) with attenuation of dose to maintain the leukocyte count between 3000 and 4000 cells/mm^3. The single-drug regimen consisted of continuous daily oral cyclophosphamide (2 mg/kg) with adjunctive radiotherapy when indicated. Radiotherapy was reserved for those patients with symptomatic areas of bulky localized disease (three instances) and was given before chemotherapy. Responders were maintained on cyclophosphamide as described above.

A complete remission (CR) was defined as a complete disappearance of all objective and

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[4] The authors wish to thank Drs. David Rothwell, Bertram Dessel, and Anthony V. Pisciotta for their cooperation.

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[6] COP = cyclophosphamide, vincristine (Oncovin), and prednisone.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2 *H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin.

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

subjective manifestations of disease for at least 1 month; a partial response (PR) was defined as a greater than 50% decrease in objective manifestations and a complete or partial disappearance of subjective manifestations for at least 1 month. Patients were excluded from this category if tumor masses grew in some areas while others regressed or if symptoms persisted such that hospitalization was still necessary.

Responses were evaluated for completeness at the end of 6 weeks of chemotherapy on either regimen. If a CR was achieved on clinical grounds, it was verified by repeat bone marrow and liver biopsies where appropriate. Patients who had a PR at the end of 6 weeks of chemotherapy were given the maintenance regimen. In several instances, continued regression occurred and at a later date a CR was achieved.

In the entire group there were almost equal numbers of lymphocytic and histiocytic cell types, but more histiocytic tumors were treated with COP than with cyclophosphamide. The numbers of well-differentiated and poorly differentiated lymphocytic tumors in each group were comparable. Thirty patients were in stage IV and half of these patients were symptomatic. There were no significant differences between the two groups in extent of extranodal distribution of disease. Nine patients had received prior radiotherapy and in two of these patients it was considered extensive. Only three patients had received prior minimal chemotherapy.

RESPONSE TO TREATMENT

The response of the entire group of patients according to histologic classification is summarized in table 1. The overall CR rate was 45% with a total response rate of 73%. There was no significant difference between the two major cell types (histiocytic and lymphocytic). However, if one distinguishes between the degree of cellular differentiation in the lymphocytic group, the patients with poorly differentiated tumors had the lowest response rate (50%). Responses according to regimen are also given in table 1. Eleven patients (55%) receiving COP had CRs compared to four patients (30%) receiving cyclophosphamide. It should be noted that two of the three patients who received adjunctive radiotherapy in the cyclophosphamide group achieved CRs as compared to two CRs and two PRs among the ten patients who did not receive radiotherapy.

Response Duration

Response duration was calculated from the time of maximal response to the time of relapse. The median duration of response for all patients with CR according to cell type is as follows: well-differentiated lymphocytic, 14+ months; poorly differentiated lymphocytic, 3 months; both lymphocytic types, 5 months; and histiocytic, 7 months. Two patients with mixed lymphohistiocytic tumors had response durations of 24+ and 29+ months respectively. The median durations of response for patients with CRs and PRs were nearly identical in both regimens: 8 and 2 months respectively.

SURVIVAL

The median survival time for the entire group from the time of diagnosis by cell type was as follows: well-differentiated lymphocytic, 21+ months (five of eight still surviving); poorly differentiated lymphocytic, 9 months (none surviving); and histiocytic, 13.5+ months (five of 15 still surviving). The median survival time for the COP group is 11.5 months with seven of 20 patients still surviving (duration ranging from 8+ to 27+ months). The median for the single-agent group is 20 months with one survivor (35+ months). The median survival time for the three patients who received radiotherapy is 30 months compared to 13 months for the non-irradiated patients.

DISCUSSION

Certain patients with non-Hodgkin's lymphomas have excellent survival rates. In a large patient population, this appears to correlate not only with clinical stage but with a nodular histologic characteristic. In our view, the distinction between nodular and diffuse histology is crucial in determining overall prognosis, even at advanced clinical stages. The purpose of this study was to evaluate the results of treatment in advanced non-Hodgkin's lymphoma where nodular histologic categories were excluded. The overall response rate of 73% (CR, 45%) illustrates the high degree of sen-

TABLE 1.—Response to therapy according to treatment regimen and histologic category

Histologic classification	COP				Cyclophosphamide				COP + cyclophosphamide			
	No. of patients treated	No. of responses			No. of patients treated	No. of responses			No. of patients treated	No. of responses		
		CR	PR	CR + PR (%)		CR	PR	CR + PR (%)		CR	PR	CR + PR (%)
Lymphocytic												
Well-differentiated									8	3	3	6 (75)
Poorly differentiated									8	4	0	4 (50)
Total	7	5	2	7 (100)	9	2	1	3 (33)	16	7	3	10 (63)
Histiocytic	12	5	5	10 (84)	3	1	1	2 (66)	15	6	6	12 (80)
Mixed	1	1	0	1 (100)	1	1	0	1 (100)	2	2	0	2 (100)
Totals	20	11	7	18 (90)	13	4	2	6 (46)	33	15	9	24 (73)

sitivity of these tumors to therapy. Our data also suggest that COP is superior to cyclophosphamide alone in producing a more complete regression of disease. It is disappointing, however, to note that in spite of continuous maintenance therapy, the median durations of remission and survival time were relatively short for both regimens. The inability to sustain long remissions with single- and multiple-drug therapy has been commented upon previously (7). It should be stressed, however, that patients in this study were not randomly assigned to the respective treatment regimens, and therefore the numbers of histiocytic versus lymphocytic tumors in each treatment group were different. This might explain the failure of the COP regimen to provide longer responses and survivals when compared to treatment with cyclophosphamide alone.

The older literature (1,8) and the data of Jones et al (2,3) suggest that the cell type and the degree of cellular differentiation influence overall prognosis. Our data for the diffuse lymphomas are generally in agreement. Patients with well-differentiated lymphocytic tumors were quite responsive and had the longest response duration and survival. Patients with poorly differentiated lymphocytic tumors were the least responsive and had the shortest response duration and survival. Patients with histiocytic tumors were also quite responsive, but although the median survival time was intermediate, the response duration was short. Strictly speaking, since there is no comparable historical control group, it is difficult to know whether the overall prognosis of diffuse lymphomas has changed over several decades. Given this limitation, it is of interest that the median total survival times according to histologic type in this study are remarkably similar to those reported by Gall and Mallory (8) 30 years ago.

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Treatment of Adenocarcinoma With Combinations of Cyclophosphamide (NSC-26271), Vincristine (NSC-67574), and 5-Fluorouracil (NSC-19893)[1,2,3]

William G. Tucker, Robert W. Talley, Montague Lane, and John D. Bonnet[4,5,6]

The theoretical purpose of combination chemotherapy is to block the metabolic cycle of the tumor cell at multiple sites and, thereby, achieve a higher tumor kill rate. Additionally, it may be possible to achieve improved antitumor effects without excessive host toxicity if the drugs are administered in combination in doses slightly lower than when they are given alone. The Southwest Oncology Group designed a study to determine if a combination of three drugs with different mechanisms of action would be additive or synergistic without increasing toxicity. The combination of cyclophosphamide, vincristine, and 5-fluorouracil (5-FU) is a modification of the program of Costanzi and Coltman (1) who treated several patients with the above regimen but added methotrexate.[7]

MATERIALS AND METHODS

A total of 196 patients with metastatic carcinoma of the breast, colon, stomach, ovary, or pancreas were entered into this study. Eligibility for admission to the protocol required a histologically proven carcinoma and measurable tumors. The patients could have received prior chemotherapy, but it was required that hematopoietic recovery had occurred, that a minimum of 21 days had elapsed following the prior therapy, and that there was proven progression of disease. In patients with breast cancer, no hormonal manipulation was permitted for at least the preceding 6 weeks and evidence of progressive disease during this 6-week interval was also required.

Patients were considered ineligible for entry into the study if (*a*) their expected survival was less than 3 months, (*b*) they had myelophthisic anemia, (*c*) the white blood cell (wbc) count was less than 2000 cells/mm^3 and/or the platelet count was less than 100,000 platelets/mm^3, or (*d*) radiation (dosage, >3000 rads) had been administered to each of two or more major bone marrow sites, ie, pelvis, mediastinum, ribs, or lumbar spine.

The planned treatment program consisted of three complete courses. However, any patient who received one complete course (4-day course of chemotherapy) and survived to start the second course of chemotherapy has been included as an evaluable patient. The reasons for non-evaluability were insufficient data, loss to followup, less than one course of chemotherapy, or a major deviation in the treatment program.

Patients with metastatic carcinoma of the

[1] Received Feb 27, 1974; revised Oct 7, 1974; accepted Nov 1, 1974.

[2] Supported by Public Health Service grants CA-12644, CA-04915, CA-03392, and CA-10187 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Cyclophosphamide: CAS reg. No. 6055-19-2; 2 *H*-1,3,-2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1); Oncovin.

5-Fluorouracil: CAS reg. No. 51-21-8; 5-FU.

All drugs were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[4] This study was done by the Southwest Oncology Group. The following investigators participated in the study: William G. Tucker (Borgess Hospital, Kalamazoo, Mich); Robert W. Talley (Henry Ford Hospital, Detroit, Mich); Montague Lane (Baylor College of Medicine, Houston, Tex); and John D. Bonnet (Scott and White Clinic, Temple, Tex).

[5] The authors wish to thank Mrs. Joan Stroud for her help in the preparation of this paper.

[6] *Reprint requests to:* William G. Tucker, MD, Borgess Hospital, 1521 Gull Rd, Kalamazoo, Mich 49001.

[7] Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[p-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-.

breast or colon were randomly assigned to the therapeutic regimens. The dose levels were 150 mg of cyclophosphamide given intravenously (iv) on Days 1 and 4, 1.0 mg of vincristine given iv on Days 2 and 4, and 300 mg/m² of 5-FU given iv on Days 1–4. Vincristine was reduced to 0.5 mg for patients over 65 years of age. The CON-FU regimen consisted of cyclophosphamide, vincristine, and 5-FU; the C-FU regimen consisted of cyclophosphamide and 5-FU; and the ON-FU regimen consisted of vincristine and 5-FU.

The dosages of drugs were reduced when moderate hematologic toxicity appeared (wbc count >1000 but <2000 cells/mm³). Cyclophosphamide was reduced to 100 mg on Days 1 and 4 and 5-FU at a dose of 300 mg/m² was administered on Days 1–3 only. Vincristine was discontinued when neurotoxicity developed (ileus or severe obstipation, severe muscular weakness, marked paresthesias, or motor deficits).

Courses of chemotherapy were repeated every 21 days if the patient recovered satisfactorily from the toxicity. A *regression* was defined as a decrease in the size of a lesion by more than 50% of the product of two diameters lasting for at least 60 days. *No change* was defined as a lesser regression without evidence of increasing disease. *Disease progression* was defined as a 25% increase in the product of two diameters or an increase in the number of lesions.

RESULTS

Carcinoma of the Breast

One hundred patients with carcinoma of the breast were included in the study. Seventy-four patients were considered evaluable. The CON-FU regression rate was 26.1%, the C-FU regression rate was 52.2%, and the ON-FU regression rate was 53.6%. The number of courses and the total doses administered were not significantly different. The regression rate and the mean duration of regression were not statistically different for patients receiving CON-FU, C-FU, or ON-FU. The longest responses were obtained in patients with bone lesions.

The minimal toxic effects observed showed no correlation with regression or progression. Neurotoxicity was evident in patients receiving the combinations with vincristine. Alopecia occurred more frequently in the groups receiving vincristine and cyclophosphamide.

Carcinoma of the Colon

Sixty-one patients with carcinoma of the colon were entered in the study and 53 were evaluable. The regression rates were 16.7% for CON-FU, 17.7% for C-FU, and 22.2% for ON-FU.

Carcinoma of the Stomach, Ovary, and Pancreas

Three patients with carcinoma of the stomach had regressions, two of them for an average duration of 4 months from treatment with CON-FU. One of four evaluable patients with carcinoma of the ovary had a regression of 6 months' duration. Ten patients with carcinoma of the pancreas were entered; one had a 2-month regression and five received only one course of therapy.

DISCUSSION

It is difficult to account for the differences in regression rates between the CON-FU, C-FU, and ON-FU regimens in the breast cancer patients. There were no more reported toxic effects with CON-FU than with C-FU or ON-FU. The number of courses administered were similar. The possibility that the third drug blocked the effectiveness of one of the other two cannot be supported in view of the results reported with the same combination or with the addition of methotrexate (1–4).

The effectiveness of the three regimens in carcinoma of the colon is no greater than the effectiveness with 5-FU alone. The numbers of patients entered with carcinoma of the stomach, ovary, or pancreas were small and the effectiveness of the combination again was not an improvement over that obtained with 5-FU alone.

It is of interest to note that four of eight patients with breast cancer metastatic to the bone had regressions while receiving ON-FU. The mean duration of these regressions was 12.5 months, and, although only three such patients were entered in the C-FU regimen, one did have a regression of bone lesions for 13

months. Of ten patients with breast cancer and primarily liver metastases, six had regressions. There is essentially no difference in the regression rate between the CON-FU, the C-FU, and the ON-FU programs; each regimen resulted in at least a 50% regression of liver metastases and a 33% regression of lung metastases. With minimal toxicity and little in the way of complications of administration, good regressions can be obtained in patients with breast cancer having visceral disease by the use of the C-FU or the ON-FU regimen.

From this study, it is evident that patients with carcinoma of the breast may be treated with minimal toxicity and achieve a 50% regression using C-FU or ON-FU. This is comparable to the regression rate of the four- and five-drug regimens of Costanzi and Coltman (1), Ansfield et al (3), and Hoogstraten.[8] Because of the neurotoxicity involved with the ON-FU regimen, the C-FU regimen would be the combination of choice. This regimen could be utilized by physicians who have minimal experience with chemotherapeutic agents and their complications with effectiveness and relative safety.

The effectiveness of the four-drug regimen reported by Costanzi and Coltman (1) of cyclophosphamide, vincristine, methotrexate, and 5-FU in breast carcinomas is essentially the same as that reported by Ansfield et al (3) and Hoogstraten [8] with the addition of prednisone [9] to the four-drug combination. The effectiveness of adding a hormonal agent to a chemotherapeutic regimen may be exhibited in the improved quality of the regression as well as the increase in the mean duration of response. In a randomized study (5), the addition of medroxyprogesterone acetate [10] to the four-drug regimen produced a mean regression of 14.5+ months as opposed to a 10-month mean regression when prednisone was added. The regression rate is not statistically different between the medroxyprogesterone acetate and the prednisone combinations; however, the quality and duration of the regression are improved when medroxyprogesterone acetate is used. It seems unlikely that the percentage of regressions with the four-drug regimen is improved over the percentage with cyclophosphamide and 5-FU, and, therefore, it would be appropriate to conduct a three-arm study of (*a*) C-FU, (*b*) C-FU plus prednisone, and (*c*) C-FU plus medroxyprogesterone acetate. This proposed protocol would answer the question of additive effectiveness of prednisone or medroxyprogesterone acetate with a safe, easily managed combination of chemotherapeutic agents with effectiveness at least equal to the four- and five-drug regimens now in use.

[8] Hoogstraten B. Southwest Oncology Group protocol: combination and single drug therapy in breast cancer, phase III. Direct communication.

[9] Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

[10] Medroxyprogesterone acetate: NSC-26386; CAS reg. No. 71-58-9; pregn-4-ene-3,20-dione, 17-hydroxy-6α-methyl-, acetate.

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Treatment of Metastatic Testicular Carcinoma With Bleomycin (NSC-125066) Used Alone or in Combination With CCNU (NSC-79037)[1,2,3]

Brief Reports and Preliminary Communications

Roland W. Sonntag, Kurt W. Brunner, Klaus Batz, and Hans Jörg Ryssel [4,5]

Our observations of definite clinical response in two patients with metastatic testicular cancer treated in a phase I study with bleomycin (1) as well as those of others (2) had prompted us to pursue this form of therapy further. We have treated an additional 18 patients who had metastatic testicular tumors with bleomycin alone or in combination with CCNU. CCNU was added in the hope of preventing cerebral metastases. Previous experience has shown this to occur relatively frequently in patients responding to treatment with other drugs incapable of reaching therapeutic concentrations in the brain by systemic administration. Because the toxic effects of CCNU and bleomycin are entirely different, both drugs could be applied in full dosage. A possible increase in remission rate or duration was also anticipated.

PATIENTS

A total of 20 patients were treated; four were treated with bleomycin alone and 16 were treated with bleomycin plus CCNU. Three patients were not evaluable; one was lost to followup, another received simultaneous radiotherapy, and the third was treated prophylactically. Another three patients could only be evaluated for the effect of induction therapy because they underwent thoracotomy for pulmonary metastases after 4–8 weeks of treatment.

The 17 evaluable patients had a median age of 28 years (18–45) at the time treatment was started. The median time from diagnosis to initiation of bleomycin or bleomycin plus CCNU therapy was 9.5 months (1 week to 30 months).

Two patients were previously treated with chemotherapy after nonradical lymphadenectomy. Definite tumor progression occurred in these two patients after 3 and 7 months. During previous chemotherapy, four other patients had had partial remissions which lasted from 2 to 4 months before tumor progression recurred and treatment with bleomycin plus CCNU was started. Five patients did not respond to previous chemotherapy and six others had never received cytostatic agents. All tumors showed objective progression at the time of therapy onset.

The histologic types of tumors treated are given in table 1.

DRUG ADMINISTRATION

CCNU was given in a single oral dose of 100 mg/m^2 and was repeated every 6–8 weeks as the leukocyte and platelet counts permitted.

Bleomycin was given twice weekly intravenously in a dose of 0.5 mg/kg (30–45 mg). With the appearance of toxicity, the dose was reduced. Patients attaining tumor remission without toxic manifestations were given a reduced dose of 30 mg every 2 weeks until a total dose level of 300 mg was reached. Subsequently, the dose was further reduced to 30 mg once monthly as long as remission lasted.

[1] Received Mar 19, 1974; revised Nov 29, 1974; accepted Dec 19, 1974.

[2] Supported in part by the Solothurn Cancer Society, Switzerland.

[3] CCNU: CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-. Supplied by Mr. P. W. Klaaborg of Lundbeck Inc., Dübendorfstrasse, Zürich, Switzerland, who is a representative of Lundbeck Pharmaceuticals, Copenhagen, Denmark.

[4] Division of Medical Oncology, Inselspital, University of Bern, Switzerland.

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TABLE 1.—Testicular cancer treated with bleomycin alone and bleomycin plus CCNU—histology and results

Histology	Total No. of patients	No. of patients with— Complete remission	Partial remission	No change	Progression
Embryonal cell carcinoma with or without seminoma	5	2	2	1	
Embryonal teratoma plus embryonal cell carcinoma or chorionepithelioma	6		3		3
Embryonal teratoma with or without adult teratoma	3		1	1	1
Chorionepithelioma	1		1		
Embryonal cell carcinoma plus chorionepithelioma	2		1	1	
Totals	17	2	8	3	4

RESULTS

Complete remission was considered as a complete disappearance of all clinically and/or radiologically measurable tumor masses for over 1 month. If initially positive, the 24-hour urinary choriogonadotropin titer must also have reverted to negative. A partial remission was defined as at least a 25% decrease in the product of two diameters of clinically or radiologically measurable tumor masses lasting for at least 1 month without the appearance of any new tumor manifestations. No change represented a diminution in tumor size of less than 25% or no measurable tumor progression for 1 month. Progression was defined as an increase in tumor size or the appearance of new tumor manifestations during treatment.

The objective tumor responses are summarized in table 1. Treatment with bleomycin alone produced one complete and one partial remission. One patient had stabilization of tumor growth for over 4 months and one patient showed tumor progression.

Of the 13 patients treated with the combination of bleomycin plus CCNU, there was one complete remission, seven partial remissions, and two patients with no change. Three patients had progressive disease during therapy.

All patients with objective tumor remission also had definite subjective improvement. Symptoms either diminished or disappeared completely.

The response durations are given in table 2. One patient has been in a complete remission for over 28 months after treatment with bleomycin alone. The second complete remission was with the combined bleomycin plus CCNU therapy and lasted 9 months. In contrast, the partial remissions were of much shorter duration with a median rate of 2.5 months (1–4 months).

TOXIC EFFECTS

The four patients treated with bleomycin alone had no hematologic toxic reactions. The leukopenia and thrombocytopenia observed in patients treated with the bleomycin plus CCNU combination were not problematic. Only one

TABLE 2.—Testicular cancer treated with bleomycin alone and bleomycin plus CCNU—remission durations

Remissions	No. of patients	Duration (mos)
Complete	2	9.0, 28+
Partial	8	1, 1.5+, 2.5, 2.5, 3, 3.7, x*, x*

*Patients undergoing thoracotomy for residual pulmonary disease; drug-induced remission duration is not evaluable.

patient had a white blood cell count below 1500 cells/mm³. The lowest platelet count observed was 42,000 platelets/mm³. However, all patients experienced skin changes due to bleomycin. These changes were never severe because dosages were reduced or the drug was temporarily omitted if these manifestations became symptomatic. Fever, frequently associated with chills, was observed in eight patients. Stomatitis was seen four times and significant hair loss five times. Nausea and vomiting were surprisingly seldom seen and were never severe; they occurred in eight patients after administration of CCNU as well as after bleomycin administration. Fifteen of the 17 patients had pulmonary metastases and therefore had chest roentgenograms regularly, at least every 4 weeks. No patients had evidence of pulmonary toxic effects associated with bleomycin even though the total dosage approached 1.0 g (900–965 mg) for three patients during maintenance therapy.

DISCUSSION

Seventeen patients with far-advanced metastatic testicular cancer of various histologic types were treated with bleomycin alone or in combination with CCNU. Eleven of these patients were resistant to other forms of chemotherapy, usually actinomycin D and vinblastine,[6] and had shown tumor progression during such treatment. Despite this, a significant objective tumor regression was attained in ten (59%) of these 17 patients.

The subjective improvement of symptoms associated with objective tumor regressions or stabilization of tumor growth was most gratifying. The toxic side effects of this therapy were not severe and in no way distracted from the benefit afforded.

It would appear that all histologic tumor types respond to treatment. No pure seminomas were treated. Neither the time from diagnosis to institution of therapy nor the type of previous treatment influenced the response to bleomycin and CCNU therapy.

Patients were not randomized for treatment with bleomycin alone or in combination with CCNU. Only four patients were treated with bleomycin alone. The superiority of combined treatment is therefore not proven. However, only one of 17 patients in our study exhibited cerebral metastases and this occurred 6 months after therapy had been discontinued. The response rate of 62% in our 13 patients treated with bleomycin plus CCNU is definitely superior to the 32% response rate reported by Blum et al (3) and Agre[7] in 37 patients treated with bleomycin alone. It, however, does not approach the 90% reported by the same authors with bleomycin and vinblastine. Also, the complete remission rate of 15% was twice that of our rate of 7.6% with bleomycin plus CCNU.

The quality of remission was generally good. Despite evidence of residual disease, many of the patients were temporarily able to resume normal activity. The main problems, therefore, lie in the low rate of complete remissions and the inability to maintain partial remissions over longer time periods. Perserverance with high bleomycin doses is not likely to afford much aid in this regard, as skin toxic reactions would be prohibitive. Based on the experience of Livingston et al (4) in lung cancer, we are now attempting to improve our results with the addition of vincristine[8] to the CCNU plus bleomycin regimen.

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[6] Actinomycin D: NSC-3053.
Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate.

[7] Agre K. Clinical summary of bleomycin. Bristol Laboratories, Apr 1971. These data are on file with CTE, DCT, NCI.

[8] Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

Clinical Trial of Bleomycin (NSC-125066) in the Treatment of Metastatic Renal Carcinoma[1,2]

Douglas E. Johnson, Roman A. Chalbaud, Paul Y. Holoye, and Melvin L. Samuels[3,4]

SUMMARY

This study had the limited aim of ascertaining the efficacy of bleomycin in controlling metastatic renal carcinoma. Fifteen patients received bleomycin intramuscularly and five patients received the drug via continuous infusion. Only two patients had responses, both mixed. We concluded that the therapeutic value of bleomycin in the treatment of advanced renal carcinoma is negligible.

[Cancer Chemother Rep 59:433–435, 1975]

Metastatic renal cell carcinoma has been proven to be highly resistant to chemotherapy and radiation therapy. Woodruff et al (1), in reviewing clinical trials with alkylating agents, periwinkle alkaloids, and 5-fluorouracil,[5] noted only occasional responses, none being complete. Prout (2) recently quoted a report by Rhamy in which vinblastine[6] produced 24 responses in 75 trials; however, no details were given as to the type of response, the duration, or the length of patient survival. NSC-122819,[7] an epipodophyllotoxin derivative classified as a spindle poison, was shown by the European Organization for Research on the Treatment of Cancer to be inactive in renal cell carcinoma but active in bladder cancer (3). Adriamycin (NSC-123127) has also been reported to have no activity in renal carcinoma (4). Medroxyprogesterone[8] has a reported response rate of 18% but the remissions it produces are usually of short duration, lasting only 4–6 months (5,6). Bleomycin, however, may be quite different. Agre[9] reported seven objective responses in 28 patients and, if it can be confirmed, this observation would make bleomycin a logical starting point for combination chemotherapy studies.

MATERIALS AND METHODS

Twenty unselected patients with metastatic renal cell carcinoma were entered into this study between June 1, 1972, and December 1, 1973. The two requirements for admission into the study were: (*a*) the leukocyte count had to be above 5000 cells/mm^3 and the platelet count had to be above 150,000 platelets/mm^3; and (*b*) there had to be measurable metastatic disease. The rating of symptoms and perform-

[1] Received July 15, 1974; revised Sept 3, 1974; accepted Sept 24, 1974.

[2] Bleomycin was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute (NCI).

[3] Section of Urology (Drs. Johnson and Chalbaud), Department of Medicine (Drs. Holoye and Samuels), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute at Houston.

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[5] 5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU.

[6] Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate.

[7] NSC-122819: 4-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside); VM 26.

[8] Medroxyprogesterone: NSC-26386; CAS reg. No. 71-58-9; pregn-4-ene-3,20-dione, 17-hydroxy-6α-methyl-, acetate; Provera.

[9] Agre K. Overview of clinical evaluation of bleomycin in the United States. Presented at New Drug Seminar, NCI, National Institutes of Health, Bethesda, Md, Feb 15, 1974.

ance status was as follows: 0 = no symptoms present, patient working full-time; 1 = symptoms present, patient fully ambulatory but unable to work full-time; 2 = patient confined to bed less than 50% of the time; 3 = patient confined to bed more than 50% of the time; and 4 = patient bedfast. The patient status information is summarized in table 1. Objective tumor response was determined by serial measurements. The grading of response was as follows: complete response = all measurable tumor disappeared; partial response = a 50% decrease in the products of the maximum diameter and the perpendicular maximum diameter; mixed response = one or more tumor masses showed partial response while other measurable disease remained unchanged or showed less than partial response; and no response = no change or continued growth.

Patients were stratified into four groups by presentations as follows: visceral = metastases to lung and/or liver; skeletal = multiple lytic metastasis; renal bed = recurrences in renal fossa, postnephrectomy status; and lymph node = lymph node metastasis.

Fifteen patients received bleomycin twice weekly by the intramuscular route. Those patients less than 60 years of age with normal pulmonary function received 30 units twice weekly for ten doses. If this course was well tolerated, a second course was given in 4–6 weeks, administering 15 units twice weekly for ten doses. Patients over age 60 and those with chronic obstructive pulmonary disease with a resting oxygen pressure of less than 60 mm Hg received 15 units twice weekly for eight to ten doses. A second course was given if there was no significant change in spirometry or blood gases. Five patients received bleomycin by continuous infusion. Fifteen units were diluted in 1000 ml of 5% glucose and distilled water and were given over a 12-hour period continuously for 10 days (total dose, 300 mg). Additional courses were not given.

TABLE 1.—Patient information

No. of evaluable patients	20
No. of women	3
Mean age (yrs, range)	58, 42–78
No. of patients receiving prior therapy	
Medroxyprogesterone	2
Radiation	2
Surgical*	14
Mean symptom status†	1.2

*Nephrectomy.
†*See* text, Materials and Methods.

RESPONSE AND TOXICITY

Table 2 summarizes the response according to type of metastasis. In the group with visceral metastases (17 patients), two showed mixed responses, both of which were brief. Although there were no responses in the remaining groups, one patient with mediastinal and supraclavicular node metastases is still alive and well 24 months after starting treatment with bleomycin. He received two courses of intrapleural 5-FU for recurrent pleural effusion with excellent control.

Toxic manifestations are summarized in table 3. In one patient, a 60-year-old Caucasian man, bleomycin pneumonitis developed 2 weeks after he had received a total dose of 165 mg. Baseline spirometry prior to bleomycin had revealed a moderately severe depression of the forced expiratory volume at 1 second (FEV_1) to 40% of the predicted, which is consistent with chronic obstructive pulmonary disease. Corticosteroid therapy resulted in a partial clearing of the infiltrates at 4 months. However, pulmonary hypertension with cor pulmonale has supervened and physical activity remains severely limited. The mean FEV_1 for the entire group was 74% predicted.

DISCUSSION

We were unable to confirm the activity of bleomycin in renal cell carcinoma. Bleomycin, given via continuous infusion, has been reported by Cvitkovic et al (7) to be more effective in cancer of the head and neck and carcinoma of the cervix than intermittent therapy, but we could not confirm this finding in our present study. Studies have shown that renal cell carcinoma can exist in a slow-growing low-growth fraction status with a doubling time of about 8 months or a rapid-growing high-growth fraction status with a doubling time

TABLE 2.—Metastasis and response

Metastasis*	No. of patients	Total dose of bleomycin (mg)	No. of responses	No. of patients still alive	Survival in mos since—	
					Diagnosis	Chemotherapy
Visceral	17	300†	2‡	4	15+§ (3–90)	5+§ (1.5–24)
Skeletal	1	225	0	0	12.5	7.5
Renal bed	1	300	0	0	7	3
Lymph node	1	180	0	1	33+	24+

*See text, Materials and Methods.
†Mean.
‡Mixed response.
§Median and range.

TABLE 3.—Summary of toxic manifestations

Toxic effect	No. of patients
Fever	3
Sclerotic skin changes of fingers	5
Polyarthralgia	2
Alopecia	4
Stomatitis	4
Interstitial pneumonitis	1
Myelosuppression	0

of 1.3 months (8). In our experience, younger patients, below age 50, usually are afflicted with the rapid-growing tumors which are generally resistant to chemotherapy and medroxyprogesterone. Thus, hormonal management should be reserved initially for elderly patients with slowly progressive disease so as not to deny them an opportunity for partial remission. Phase II studies should be considered immediately for those who don't respond to medroxyprogesterone as well as for younger patients.

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Cancer Chemotherapeutic Response and Intravenous Hyperalimentation[1,2,3]

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Brief Reports and Preliminary Communications

To date, no data have shown enhancement of chemotherapeutic response by concomitant intravenous hyperalimentation (IVH); however, previous studies (1) are derived from heterogeneous populations with respect to variables influencing response. A recent chemotherapy protocol for bronchogenic carcinoma (oat cell excluded) affords partial control of variables by providing a homogeneous population of patients sharing a similar diagnosis, extensive disease category, and identical treatment. Of 30 patients evaluable for chemotherapeutic response, ten required IVH in order to initiate aggressive chemotherapy. This report constitutes a retrospective review of the patients treated according to this recent chemotherapy protocol to determine if the probability of response to chemotherapy was related to concurrent IVH.

MATERIALS AND METHODS

Thirty-eight patients with bronchogenic carcinoma (oat cell excluded) were registered for chemotherapy with bleomycin (15–30 mg/day by continuous intravenous [iv] infusion, Days 1–4), cyclophosphamide (200 mg/m² iv, Days 6–10), vincristine (2 mg iv, Days 6 and 9), methotrexate (20 mg/m² iv, Days 6 and 9), and 5-fluorouracil (450 mg/m² iv, Days 6–10). The cycle was repeated every 29 days. All patients had extensive disease (according to the Veterans' Administration Cooperative Lung Study Group criteria) (2) but otherwise were acceptable for chemotherapy regardless of general condition or nutritional status. Thirty patients (13 with adenocarcinoma, 11 with squamous cell carcinoma, and six with poorly differentiated carcinoma) were evaluable for chemotherapeutic response (50% reduction in the product of two perpendicular diameters of all measurable lesions). Eight nonevaluable patients included four who died less than 29 days after the start of treatment and four with improved but poorly evaluable radiographic lesions. None of the nonevaluable patients received IVH.

IVH was administered as described by Dudrick et al (3) through a subclavian vein catheter. Each liter of solution (1000 kcal) contained 25% glucose and 4.25% crystalline amino acids. Ten debilitated patients received IVH at 2000 kcal/day (mean, 22 days; range, 9–41 days) initiated during the first cycle of chemotherapy. Patient selection was based entirely on apparent nutritional need.

Percent weight loss prior to chemotherapy was derived from the patient's stated usual weight and the recorded weight on the day chemotherapy was started. Patients were placed into high weight loss (>6%) and low weight loss (<6%) categories based on the observation that the least prior weight loss for an IVH patient was 6.5%. Thus, the evaluable patients

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[2] Supported in part by Public Health Service grant CA-05831 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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were divided into three subcategories: high weight loss-IVH, high weight loss-nonIVH, and low weight loss-nonIVH.

The four patients who died in less than 29 days and the four patients with poorly evaluable lesions had prior weight losses of 0, 7.7%, 8.8%, 17.7%, questionable, 5.7%, 7.6%, and 10.6% respectively.

RESULTS

There were 11 responders to chemotherapy (four of 13 patients with adenocarcinoma, four of 11 with squamous cell carcinoma, and three of six with poorly differentiated carcinoma) (table 1). Six of eight low weight loss patients responded compared to five of 22 high weight loss patients (P = 0.015, one-tailed test). IVH patients had a higher response rate (five of ten patients) than nonIVH patients (six of 22 patients), but the difference is not significant (P =0.250). However, by restricting attention to high weight loss patients it is seen that five of ten patients in the high weight loss-IVH group responded (two of six with adenocarcinoma, one of two with squamous cell carcinoma, and two of two with poorly differentiated carcinoma) compared to none of 12 patients in the high weight loss-nonIVH group (P = 0.010). No prognostic factors favoring IVH patients were observed (table 2). High weight loss-IVH and high weight loss-nonIVH patients each received a median of three courses of chemotherapy at fixed dosage schedules.

No catheter-related septic complications were encountered, and no objective differences in gastrointestinal or hematologic toxicity (me-

TABLE 1.—Thirty patients evaluable for chemotherapeutic response

Treatment group	Patient No.	Weight loss (%)	Chemotherapy response
IVH	1	6.5	Yes
	2	8.2	No
	3	10.7	No
	4	13.3	Yes
	5	17.2	Yes
	6	18.6	No
	7	19.4	No
	8	19.6	Yes
	9	21.7	Yes
	10	23.7	No
NonIVH*	11	0.0	Yes
	12	0.3	Yes
	13	0.8	Yes
	14	1.8	Yes
	15	1.9	Yes
	16	2.7	No
	17	3.7	No
	18	5.7	Yes
	19	6.5	No
	20	7.3	No
	21	8.1	No
	22	9.2	No
	23	9.9	No
	24	12.8	No
	25	13.0	No
	26	13.8	No
	27	14.2	No
	28	14.7	No
	29	16.1	No
	30	16.8	No

*No response in nonIVH group beyond 5.7% weight loss.

TABLE 2.—Comparison of variables possibly affecting chemotherapeutic response (high weight loss patients only)

Variables	IVH (10 patients)	NonIVH (12 patients)
Mean age, yrs (range)	58.4 (43–77)	59.3 (44–68)
Mean % weight loss (range)	15.9 (6.5–23.3)	11.9 (6.5–16.8)
Mean symptom status* (range)	2.7 (1–4)	1.2 (1–2)
Histology		
Squamous cell	2	6
Adenocarcinoma	6	4
Poorly differentiated	2	2
No. of patients who received previous therapy	6 (5 radiotherapy; 3 chemotherapy)	3 (3 radiotherapy; 1 chemotherapy)

*Symptom status: 0 = no symptoms, fully active; 1 = has symptoms, fully ambulatory; 2 = bedridden <50% of the time, requires nursing assistance or equivalent; 3 = bedridden >50% of the time; 4 = bedfast.

dian white blood cell count nadir 1800 cells/mm^3) were observed between IVH and non-IVH patients.

DISCUSSION

The data suggest that chemotherapeutic response is most probable in patients with stable body weight and that increased responsiveness might be realized by concomitant IVH of patients who are losing weight. Law et al (4) have provided evidence that nutritional status is correlated with immunocompetence and that lymphocyte phytohemagglutinin reactivity is enhanced in cachectic patients after 18 days of IVH at 2000–3000 kcal/day. On the other hand, Meyer (5) has suggested that relative tumor cell reliance on glycolytic metabolism be exploited by providing excess substrate in the form of glucose during chemotherapy, thus inducing intracellular lactic acidosis and tumor cell vulnerability. The observations of the present study provide the basis for the prospective testing of the hypothesis that chemotherapeutic response in bronchogenic carcinoma may be favorably influenced by IVH of patients who are losing weight.

LIST OF COMPOUNDS

Bleomycin: NSC-125066

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Immediate Cerebral Symptoms During Rapid Intravenous Administration of Cyclophosphamide (NSC-26271)[1,2]

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Brief Reports and Preliminary Communications

Although "dizziness of short duration" (1) and "facial flushing and a feeling of faintness" (2) have been reported by patients receiving a rapid intravenous (iv) administration of cyclophosphamide, central nervous system manifestations (aside from nausea and vomiting) have not received much attention. In the past year, five patients have been observed who have regularly experienced peculiar sensations during the infusion of cyclophosphamide.

The drug concentration was 20 mg/ml, the diluent was sterile distilled water, the usual dose was 500 mg administered by iv push within 3 minutes, and the dosage schedule was usually once every 1 or 2 weeks. All five patients have reported sensations while the needle was still in place, usually by the time 250 mg had been administered. These sensations were noted with the first injection of cyclophosphamide and were readily reproduced with subsequent injections. They were nearly identical in quality and degree in each patient. The symptoms were variously described (*see* table 1), but most patients compared them to the effects of alcoholic intoxication; they were usually evanescent and disappeared within a few minutes. All patients encouraged the completion of the injection, returned for further treatment, and described the reactions as tolerable, except for the first patient who expressed a preference for a change in therapy after the initial series of injections. He was the only one to exhibit a facial flush and diaphoresis on several occasions.

Arena (3) has reported an oropharyngeal sensation that was noted after cyclophosphamide administration which is measurable within seconds after the injection. The similarity of

TABLE 1.—Sensations reported by patients after rapid iv infusion of cyclophosphamide

Patient age (yrs)	Sex	Racial extraction	Cancer diagnosis	Symptoms	No. of injections
44	M	Japanese	Lymphoma	Dizzy, faint, anxious	8
45	F	Caucasian	Lymphoma	"High," "warm," "like alcohol"	8
65	M	Caucasian	Lymphoma	Dizzy, "drunk"	9
53	F	Caucasian	Bronchus (oat cell)	"High," crying spell, "like I had a drink"	7
60	F	Chinese	Colon	"Unreal," pleasant, relaxed feeling	2

[1] Received Aug 7, 1974; accepted Sept 26, 1974.

[2] Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

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the symptoms in the present series to alcoholic intoxication is of interest because of other properties previously reported such as metabolism by microsomal enzyme systems (4), effects on water metabolism (5,6), and identification of aldehydic compounds as important metabolites of cyclophosphamide (4). The rapid onset of these cerebral symptoms suggests that they are attributable to the direct effects of the parent compound.

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Streptozotocin (NSC-85998) in the Treatment of Lymphoreticular Malignancies[1]

M. J. O'Connell [2,3] and P. H. Wiernik [2,4]

Streptozotocin, a nitrosourea antibiotic with activity against murine leukemia (1), has been shown to have antitumor activity against certain human neoplasms including pancreatic islet cell carcinoma (2), carcinoid tumor (3,4), Hodgkin's disease (5), and other miscellaneous tumors (4,6). This agent is of particular interest because it is not usually myelosuppressive in doses which have been shown to have activity against human tumors (2–6). Thus, it would be of interest to document antineoplastic activity in additional neoplasms, since this agent could potentially be used in patients with active disease who have bone marrow suppression from radiation therapy and/or chemotherapy. In addition, streptozotocin might be of use in combination chemotherapy regimens because of its bone marrow sparing effect, as well as the fact that its major toxicity (nephrotoxicity) is not shared by many of the other commonly used chemotherapeutic agents.

Our preliminary experience with streptozotocin given to patients with a variety of malignant tumors has been included in a previous report (5). However, we have subsequently had additional experience using streptozotocin in the treatment of malignant lymphoma and acute lymphocytic leukemia. We wish to report here our overall experience with streptozotocin in these diseases.

PATIENTS AND METHODS

All of the patients included in this report had received extensive therapy prior to the initiation of streptozotocin and were refractory to conventional chemotherapeutic agents. All patients had advanced disease and all except one have subsequently died as a result of their malignancy. Many of the lymphoma patients had received extensive radiation therapy in addition to chemotherapy.

Patients were treated with either daily intravenous (iv) doses of 0.5 g/m² of streptozotocin for 5 consecutive days with courses repeated at 2-week intervals (regimen A), or weekly doses, usually 1.0–1.5 g/m²/dose iv (regimen B). Therapy was to continue for 1 month unless there was clinical deterioration which was felt to require a change in therapy by the attending physician.

Serial hemograms, liver function studies, renal function studies including urinalysis, and measurement of tumors when present were carried out for all patients.

RESULTS

Table 1 summarizes the responses observed. All patients had some degree of nausea and vomiting, although this was not a limiting factor in drug administration for any patient. Transient proteinuria was observed in several cases. In addition, three patients had evidence of renal failure, but in each case there was extensive infiltration of the kidneys by malignant cells, and one of these patients also inadvertently received excessive doses of gentamicin. There was no evidence of myelosuppression resulting from the doses of streptozotocin employed in this study.

Of the nine patients who had acute lymphocytic leukemia, only one showed any evidence of antitumor effect. He was a 26-year-old man who had disappearance of blast cells from his peripheral blood and stabilization of his clinical course with no need for blood transfusion for a period of 3 months. The remaining patients

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TABLE 1.—Streptozotocin in lymphoreticular malignancies

Disease	Patient No.	Age/sex	Dosage regimen*	Total dose (g)	No. of days on study	Response†	Toxicity‡
Poorly differentiated lymphocytic lymphoma	1	61/M	A	11.3	68	PR	None
	2	50/M	B(1.0–1.5)	23.9	82	PR	Transient proteinuria
	3	50/M	A	17.7	68	IMP	Severe anorexia
	4	67/M	B(1.0)	7.4	29	IMP	None
	5	43/F	B(1.5)	6.9	21	DP	None
	6	53/M	B(1.5–3.0)	14.6	24	NR	Trace proteinuria
	7	36/M	B(1.0–1.5)	9.9	35	NR	None
Histiocytic lymphoma	8	52/M	B(1.5)	6.4	26	DP	None
	9	28/F	B(1.5)	5.1	15	NR	Unevaluable (renal lymphoma)
	10	42/F	B(1.5)	5.5	9	DP	Unevaluable (renal lymphoma)
Acute lymphocytic leukemia	11	26/M	B(1.0–1.5)	28.3	97	Disappearance of peripheral lymphoblasts	None
	12	21/M	B(1.5–1.75)	7.4	17	DP	None
	13	15/F	B(1.5–2.0)	6.7	21	DP	Unevaluable (gentamicin toxicity; leukemic renal infiltrates)
	14	17/M	B(1.5)	5.1	9	DP	None
	15	20/F	B(1.5)	3.9	14	NR	None
	16	27/M	A	6.0	8	DP	Transient proteinuria
	17	13/M	A	8.25	14	DP	None
	18	17/M	A	8.80	14	DP	None
	19	60/F	A	8.45	28	NR	None

*A: 0.5 g/m²/day iv × 5; B: Nos. in parentheses = g/m²/wk iv.

†PR = partial remission (>50% reduction in size of all tumor masses); IMP = objective tumor regression less than partial remission; NR = no objective response; DP = disease progression.

‡Other than nausea and vomiting.

either had no discernible response or had progressive hematologic deterioration during therapy. Two patients with poorly differentiated lymphocytic lymphoma had greater than 50% reduction in their generalized adenopathy. Two additional patients had less than 50% reduction in enlarged peripheral nodes and a retroperitoneal mass respectively. The duration of the responses observed was relatively brief, as indicated in the table. None of the three patients with histiocytic lymphomas showed evidence of antitumor effect.

COMMENT

Although we were encouraged by the hematologic improvement observed in our first patient with acute lymphocytic leukemia treated with streptozotocin, none of the subsequently treated patients have shown any effect whatsoever from this drug. Furthermore, the single patient with acute lymphocytic leukemia who did show improvement in his peripheral blood continued to have a packed marrow, and hepatic infiltration by lymphoblasts developed during streptozotocin therapy. Thus, we have abandoned this agent for the treatment of advanced, refractory acute lymphocytic leukemia at our institution.

On the other hand, two patients with advanced disseminated poorly differentiated lymphocytic lymphoma which was refractory to all conventional chemotherapeutic agents showed definite, clinically useful responses although of brief duration. Two additional patients showed some minor evidence of antitumor effect. These results suggest to us that streptozotocin may have a place in the therapy of this type of malignant lymphoma in patients who are myelosuppressed from radiation or chemotherapy or who are refractory to the usual chemotherapeutic agents. We have included streptozotocin in our current combination chemotherapy regimen for previously treated patients with malignant lymphoma refractory to alkylating agents, vinca alkaloids, steroids, and adriamycin (NSC-123127). Although none of the three patients with histiocytic lymphoma in our series showed evidence of antitumor effect, the number of patients treated was obviously too small to draw any firm conclusions.

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Single Dose Toxicity of Actinomycin D (NSC-3053) in Dogs [1,2,3]

M. C. Henry,[4] A. R. Roesler,[4] and E. DiDomenico [4,5,6]

Brief Reports and Preliminary Communications

Clinically, actinomycin D is commonly given in divided doses for 3–5 days (1). Preliminary studies in dogs have shown that this regimen is less toxic than an equivalent dose given as a single rapid injection and that emesis is more pronounced after high single doses than after lower repeated daily doses (2). Other clinical toxic effects, bone marrow depression, stomatitis, and alopecia, do not usually become apparent until 2–4 days after a course of therapy is stopped and may not be maximal before 1–2 weeks. Previous studies in this laboratory (3) have defined the toxicity produced by actinomycin D in beagles by using the clinical schedule of five daily doses. Since this schedule has recently been used in clinical investigations, the present investigations were performed to determine the detailed toxic effects of a single dose regimen.

METHODS

Actinomycin D was supplied as the clinical formulation. Two beagles were treated at each dose level given (total, eight dogs); one dog was killed at 1 week, the other was held for 45 days.

RESULTS

The toxic effects of actinomycin D on the hematologic parameters of dogs are summarized in table 1. At the 0.16-mg/kg dose level, hemoconcentration and leukopenia were noted in dog No. 548M on Day 4, whereas dog No. 647F had a normal hematocrit, lymphopenia, and a slight increase in platelet count. Clearance of sulfobromophthalein (BSP) was slightly reduced in both of these dogs as were the serum levels of sodium, chloride, and calcium. Toxic signs were present on Day 4 and included loss of body weight, emesis, anorexia, and bloody stools. Histopathologic examination revealed: congestion in the liver, brain, spleen, kidney, adrenals, and intestinal tract; hemorrhage of the adrenals, kidney, thymus, lymph nodes, bone marrow, and intestinal tract; and a marked depletion of cells in the lymphoid tissues and bone marrow.

Single doses of 0.08 mg/kg of actinomycin D produced leukocytosis on Day 2 and/or Day 4, due to a marked neutrophilia and lymphopenia; reticulocytopenia was noted on Days 4, 8, and 15. Leukopenia was observed on Day 8 with a differential shift to immature neutrophils in dog No. 539M; metamyelocytes were present in blood smears from dog No. 639F. Platelet counts, serum calcium levels, and body weight were decreased in both dogs. Increased serum alkaline phosphatase activity and reduced sodium and chloride levels were noted in dog No. 539M killed 1 week after the end of treatments. Pathologic findings included marked congestion in the spleen and liver, hemorrhages in the lung and spinal cord, marked cellular depletion in lymphoid tissue, and slight reduction of cells in the bone marrow. The tissues from dog No. 639F appeared normal 45 days after treatment.

Reductions in reticulocyte, platelet, and white blood cell (wbc) counts with a differential shift were noted in dog No. 644F after 0.04 mg/kg. Dog No. 543M had a differential shift on Day 8 (lymphocytosis) and a neutrophilia and

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[3] Actinomycin D was supplied by Drug Research and Development, DCT, NCI.

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TABLE 1.—Toxicity of actinomycin D in beagle dogs: single dose*

Dog No./sex	Dose level Mg/kg	Dose level Mg/m²	Hematocrit (%)	Leukocytes (×10³/mm³)	Mature neutrophils (%)	Lymphocytes (%)	Platelets (×10³/mm³)	Comments
548/M	0.16	3.2	64	3.5	89	10	2.00	On Day 2, normal wbc count with neutrophilia and lymphopenia in both dogs; dog No. 647 had change in wbc due to lymphopenia; death in both dogs Days 5 and 6
647/F			50	7.4	92	8	4.93	
539/M	0.08	1.6	40	5.2	25	68	1.37	Marked neutrophilia and lymphopenia prior to leukopenia in both dogs; dog No. 639 had 38% immature neutrophils
639/F			45	8.6	6	52	0.62	
543/M	0.04	0.8	44	18.8	63	36	5.40	Dog No. 543 had differential shift prior to neutrophilia
644/F			47	8.0	37	48	1.82	
547/M	0.02	0.4	47	14.1	58	35	2.18	
645/F			41	14.1	60	29	1.30	
Mean control values†			47 ±3	12.5 ±2.6	56 ±11	36 ±9	3.00 ±0.64	

*Values given are maximum changes.
†Mean values for 8 control animals ±SD.

thrombocytosis on Day 16. Dog No. 644F, who was killed 1 week after the end of treatment, had moderate medullary congestion and lymphocyte depletion of the lymph nodes and scattered foci of centrilobular congestion in the liver.

Toxicosis after 0.02 mg/kg was limited to a moderate reduction in platelet and red blood cell (rbc) counts in dog No. 645F. Histopathologic examination of tissues from dog No. 547M killed on Day 8 revealed moderate cellular depletion in the spleen, lymph nodes, and bone marrow.

DISCUSSION

The results obtained in this study using a single dose schedule were compared with a previous report from this laboratory (3) and they indicate that fractionation of the dose reduces drug toxicity. The lethal dose for single injections is 0.16 mg/kg, whereas the 0.30-mg/kg dose is lethal on a five daily dose schedule (0.06 mg/kg/day). On both dosage regimens the dogs died within 7 days. Philips et al (2) reported lethality after 0.10 mg/kg, regardless of the dose regimen. They observed 100% mortality after single doses (Day 5) and 17% after a ten-dose schedule (Day 16). The differences in quantitative toxicity between the two laboratories may be related to physiologic differences between mongrels (2) and beagle dogs (3).

Toxic effects on sensitive tissues were similar in all studies. Weight loss began after Day 2 and bloody diarrhea appeared by Day 4. Hemoconcentration, reticulocytopenia, lymphopenia, and neutrophilia (with slight change in platelet counts in most dogs) were noted. The extracellular electrolytes in serum sodium, chloride, and calcium levels were decreased. Serum potassium levels remained constant. There were some differences in manifestation of drug tox-

icity between the single and five daily dose regimens. There was a more pronounced leukopenia after single high toxic doses. Administration of the drug in multiple doses produced a greater rbc depression and there appeared to be a persistent effect on erythropoiesis. Toxic effects on renal and hepatic function were prominent after multiple injections as indicated by alterations in blood urea nitrogen, BSP, serum creatinine, serum alkaline phosphatase, and serum glutamic pyruvic transaminase levels, whereas single doses produced equivocal increases in these parameters.

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Comparative Study of Methyl-CCNU (NSC-95441) With Cyclophosphamide (NSC-26271) and 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) With Vincristine (NSC-67574) in Patients With Disseminated Malignant Melanoma [1,2,3]

Brief Reports and Preliminary Communications

David L. Ahmann, Richard G. Hahn, Harry F. Bisel, Robert T. Eagan, and John H. Edmonson [4,5]

Previous evaluations have shown that 5-(3,-3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) has significant antitumor activity in patients with disseminated malignant melanoma (1,2). Recently, we have completed the second of two studies assessing the effect of NSC-45388 in combination with vincristine in the treatment of this disease (3). This combination may or may not be more effective than NSC-45388 when used alone.

Our previous experience utilizing the nitrosoureas was initiated with a study evaluating CCNU [6] (3) which produced disappointing results. Our latest experience with a related nitrosourea, methyl-CCNU, resulted in five of 19 patients having an objective clinical regression (4). Methyl-CCNU seems to be one of the most active compounds screened against a variety of solid tumors, particularly the subcutaneously implanted B16 melanoma and the Lewis lung tumor.[7] The toxicity of this agent when used alone seems quite acceptable and less of a problem than the toxicity encountered with its related compound, CCNU.

Combination chemotherapy with cyclophosphamide plus a nitrosourea has been potentiating in its effect against transplanted murine leukemias and certain solid tumors (5).

MATERIALS AND METHODS

Forty patients (23 men and 17 women; mean age 53.9 years, median age 56 years) with disseminated malignant melanoma were included in this study. Seven patients had positive reactions for urinary melanin or its precursors as measured by nitroprusside reagents. Of the 40 patients entered in the study, 36 had visceral-dominant disease and four had only soft tissue involvement.

None of the patients entered in our study had had previous chemotherapy with any of the drugs we used in this study and only two patients had had previous chemotherapy of any kind (both of these patients had received hydroxyurea [8] previously). All patients were acceptable candidates for cytotoxic chemotherapeutic drug trials with respect to performance scale and hematologic status. All patients were randomized to one of two treatment programs.

[1] Received Sept 6, 1974; revised Nov 18, 1974; accepted Nov 20, 1974.

[2] Supported in part by Public Health Service grant CA-11911 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] Methyl-CCNU: CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

Cyclophosphamide: CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan.

NSC-45388: CAS reg. No. 4342-03-4.

Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

The authors wish to thank Dr. Stephen K. Carter, Cancer Therapy Evaluation, Division of Cancer Treatment (DCT), NCI, for supplying the chemotherapeutic agents used in this study and for his assistance.

[4] Division of Medical Oncology, Mayo Clinic and Mayo Foundation, Rochester, Minn.

[5] *Reprint requests to:* David L. Ahmann, MD, Division of Medical Oncology, Mayo Clinic and Mayo Foundation, Rochester, Minn 55901.

[6] CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

[7] Wasserman TH, Comis RL, Broder LE, et al. Clinical brochure: 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (NSC-95441, Me-CCNU), Mar 1974. Prepared by the DCT, NCI, Bethesda, Md.

[8] Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1.

Methyl-CCNU was given at a dose of 150–175 mg/m² orally on Day 1. Cyclophosphamide was also given on Day 1 at a dose of 500–800 mg/m² by rapid intravenous (iv) infusion. The dosages of the methyl-CCNU and cyclophosphamide combination were accelerated early in our study to produce adequate but clinically acceptable toxicity. NSC-45388 was given at a dose of 300–340 mg/m² adjusted for clinical risk on each of 5 successive days by rapid iv infusion; vincristine was given at a dose of 1.4 mg/m² (not to exceed 2.0 mg) on Days 1 and 5 of this schedule.

Patients receiving NSC-45388 and vincristine were reevaluated 6 weeks after the onset of treatment and those patients receiving methyl-CCNU and cyclophosphamide were reevaluated after 8 weeks.

An objective response was defined as a decrease of at least 50% in the product of the perpendicular diameters of the indicator lesion(s) which lasted a minimum of 4 weeks. Any patient whose disease had progressed despite the initial chemotherapeutic effort was treated with the alternate program on a crossover arrangement.

RESULTS AND DISCUSSION

Objective clinical regressions were observed in two of 20 patients who were exposed to NSC-45388 and vincristine as primary therapy and in one of 20 patients receiving methyl-CCNU and cyclophosphamide as primary therapy (table 1). Of 17 patients who were crossed over to the alternate treatment program upon primary treatment failure, no objective regressions occurred. This finding was consistent with studies previously reported in which secondary treatment in disseminated malignant melanoma is of little or no benefit.

The toxic effects associated with these combination programs are shown in table 2. If patients manifested vincristine neuropathy, the dose of this drug was subsequently reduced to a total of 1 mg on Days 1 and 5. Myelosuppression was more frequent and more profound in the group who received methyl-CCNU and cyclophosphamide and was more severe and prolonged when the doses of these two drugs were accelerated to 175 and 800 mg/m² respectively.

The mean duration of survival from initiation of treatment of patients receiving methyl-CCNU and cyclophosphamide was 19.7 weeks compared to 16.7 weeks for those patients receiving NSC-45388 and vincristine. No correlation was observed between the responses observed and the presence or absence of melanin or its precursors in the urine.

The combination chemotherapeutic programs employed in this study showed no improvement over the single drug programs as previously reported. The 10% and 5% response rates ex-

TABLE 1.—Results of treatment of patients with visceral-dominant disease and nonvisceral disease

Treatment	Visceral-dominant		Nonvisceral	
	No. of patients entered	No. of regressions	No. of patients entered	No. of regressions
Primary				
NSC-45388 and vincristine	18	2*	2	0
Methyl-CCNU and cyclophosphamide	18	1†	2	0
Totals	36	3	4	0
Secondary				
NSC-45388 and vincristine	9	0	0	0
Methyl-CCNU and cyclophosphamide	8	0	0	0
Totals	17	0	0	0

*16 and 31 wks duration.
†22 wks duration.

TABLE 2.—Toxic effects of primary and secondary treatments in evaluable patients

Toxic effects	Treatment	
	NSC-45388 and vincristine*	Methyl-CCNU and cyclophosphamide†
Nausea, vomiting	29	17
Diarrhea	12	5
Neurologic	10	0
Alopecia	8	6
Leukocytes‡		
≤3000 cells/mm³	6	17
≥1000 cells/mm³	0	3
Platelets		
≤100,000 platelets/mm³	3	6

*Total No. of patients = 29.
†Total No. of patients = 28.
‡Nadir = 2.3 × 10^3/mm³, median duration of 6.6 days on NSC-45388 and vincristine; nadir = 1.8 × 10^3/mm³, median duration of 9.5 days on methyl-CCNU and cyclophosphamide.

hibited in this study are well within the statistical range of those rates experienced in other studies utilizing methyl-CCNU and NSC-45388 as single agents.

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ANNOUNCEMENTS

Wisconsin Clinical Cancer Center Tenth National Conference

Madison, Wisconsin, September 11–13, 1975

The subject of the Wisconsin Clinical Cancer Center Tenth National Conference will be Systemic Treatment of Early and Advanced Cancer. For further information contact: Fred J. Ansfield, MD, Rm 701C, University Hospitals, Madison, Wis 53706.

Examination of the American Board of Internal Medicine in the Subspecialty Area of Medical Oncology

The examination of the American Board of Internal Medicine in the Subspecialty Area of Medical Oncology will be administered on Tuesday, October 21, 1975. Registration begins January 1 and ends March 15, 1975. For information and application forms please write to: American Board of Internal Medicine, 3930 Chestnut Street, Philadelphia, Pa 19104.

Oncology Placement Service

The American Society of Clinical Oncology's Clearing House of Oncologic Opportunities is accepting inquiries from applicants concerning staff positions and oncology fellowships, as well as announcements of openings for such applicants. The service is open to members and non-members of ASCO. Please direct all inquiries and announcements to Michael J. Brennan, MD, American Society of Clinical Oncology, 110 E Warren, Detroit, Mich 48201.

Fellowships in Medical and Surgical Oncology

The Divisions of Medical and Surgical Oncology at Boston University Hospital are offering 1–2-year fellowships beginning July 1975 and July 1976. Candidates must have completed residency training in medicine or surgery. The Medical Oncology Division has a new 34-bed oncology unit and the Surgical Oncology Division has a 30-bed unit. The floors are staffed by house officers and students. Joint teaching sessions are run by the Surgical Oncology, Medical Oncology, Radiotherapy, and Pathology Divisions. During the first year the fellow will spend 6–8 months on the primary service with elective rotations to the other oncology service, as well as to the hematology, pathology, and radiotherapy services. The primary emphasis in the second year in the Medical Oncology Division is in developing a research expertise in some area of oncology. Opportunities for research are numerous within our Cancer Research Center.

Please send bibliographies to: Marc J. Straus, MD, Chief, Division of Medical Oncology, or Peter Mozden, MD, Chief, Division of Surgical Oncology, Boston University Hospital, 750 Harrison Avenue, Boston, Mass 02118.

Sixth New Drug Seminar: Dimethyl Triazeno Imidazole Carboxamide

Bethesda, Maryland, April 25, 1975

The Division of Cancer Treatment of the National Cancer Institute is planning its Sixth New Drug Seminar to be held on April 25, 1975, in the Jack Masur Auditorium on the National Institutes of Health reservation in Bethesda, Maryland.

The subject of the seminar will be dimethyl triazeno imidazole carboxamide (Dacarbazine, DTIC, DIC), a new anticancer drug developed by the chemotherapy development program of the Division of Cancer Treatment. This drug will soon be commercially available. Dacarbazine has significant clinical activity against malignant melanoma, a tumor notoriously unresponsive to drug treatment. The seminar will cover the drug's initial development, mechanism of action, pharmacology, and clinical activity. Emphasis will be placed upon its role in the treatment of melanoma, both alone and in combination with other drugs, as well as with immunotherapy.

This seminar should be of interest to oncologists, internists, dermatologists, and other physicians with interests in the treatment of cancer.

For further information write to: Dr. Vincent T. DeVita, Jr., Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bldg 31, Rm 3A52, Bethesda, Md 20014.

Symposium on Human and Clinical Tumor Immunity

Brussels, Belgium, May 26–29, 1975

The Annual European Organization for Research on Treatment of Cancer Symposium will be held in Brussels at the Institut Bordet from May 26 to 29, 1975. The topic will be "Human and Clinical Tumor Immunity." The various aspects covered by European and American speakers will include: General Tumor Immunity, Immunological Tests in Cancer Patients, Tumor Immunotherapy, and New Aspects in Leukemias and Lymphomas. The fee will be 1500 Belgian Francs. There will be some free communications related to the above topics.

For information regarding the symposium please write to: Dr. Joseph Wybran, Service d'Immunologie, Hôpital Universitaire Saint Pierre, rue Haute 322, 1000 Brussels, Belgium.

CORRECTION

An error appears on page 795 of the November/December 1974 issue of *Cancer Chemotherapy Reports* in the article "Inhibition of L-Asparagine Synthetase by a New Amino Acid Antibiotic With Antitumor Activity: L-(αS,5S)-α-Amino-3-chloro-4,5-dihydro-5-isoxazoleacetic Acid (NSC-163501)" by David A. Cooney, et al. The fourth sentence under the heading *Binding Experiments* should read as follows: "Using 0.25 M lithium citrate buffer (*pH 2.72*), NSC-163501 elutes from the long column of the amino acid analyzer at a position midway between L-aspartate and L-threonine."

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